SOT | Society of Toxicology

48th Annual Meeting and ToxExpo™

Baltimore, Maryland

March 15–19, 2009

Baltimore Convention Center

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The Toxicologist

March 2008

The Toxicologist

47th Annual Meeting and ToxExpo™

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2009

48th Annual Meeting and ToxExpo™

Baltimore, Maryland

Deadline for Proposals for SOT 2009 Annual Meeting Sessions: April 30, 2008

WHY SUBMIT A PROPOSAL?

1. To present new developments in toxicology.
2. To provide attendees an opportunity to learn about state-of-the-art technology and how it applies to toxicological research.
3. To provide attendees an opportunity to learn about the emerging fields and how they apply to toxicology.

2009 Thematic Approach

The Program Committee will carry forward the thematic approach for the 2009 Annual Meeting. All proposal submissions will be reviewed for their relevance under the following themes:

- **Biomarkers**
- **Epigenetics**
- **Inflammation and Disease**
- **Neurodegenerative Disease**

Due to the timeliness of the Nanotechnology theme, it will again be highlighted for the 2009 meeting.

SESSION TYPES

Continuing Education—Emphasis on quality presentations of generally accepted, state-of-the-art knowledge in toxicology

Note: CE Courses will be held on Sunday.

Symposia—"Cutting-edge" science; new areas, concepts, or data

Workshops—State-of-the-art knowledge in toxicology

Roundtables—Controversial subjects

Historical Highlights—Review of a historical body of science that has impacted toxicology

Informational Sessions—Scientific planning or membership development

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Submit your proposal on-line at www.toxicology.org

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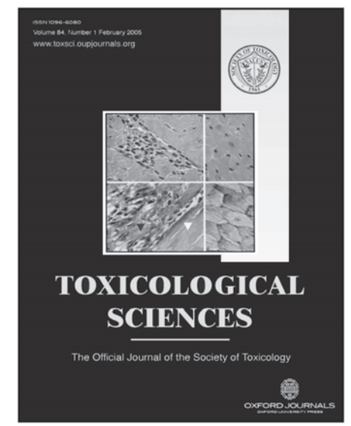
TOXICOLOGICAL SCIENCES



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OXFORD JOURNALS
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CE 1 MINI-PIGS AS AN ALTERNATIVE NON-RODENT SPECIES IN TOXICOLOGY AND SAFETY STUDIES.

G. Washer[1] and A. Makin[2]. [1]*LAB Research Inc., Montréal, QC, Canada and* [2]*Research Denmark (Scantox), Copenhagen, Denmark.*

This course will focus on the regulatory acceptability and use of minipigs as an alternative to traditional non-rodent species for non-clinical studies of pharmaceuticals. Attention will be given to their use in general toxicology and safety studies, including non-routine routes and embryofetal development studies. The choice of non-rodent species for safety assessment of new pharmaceuticals is becoming increasingly important. Researchers have adopted the paradigm that we use the dog, and if the dog is no good, then we use the primate. With the ever increasing ethical concerns relating to the use of primates, and other groups concerned with the use of man's best friend, attention has increasingly focused on the potential of the pig and minipig. An increasing appreciation of the similarities between man and the pig in structure of skin, gastrointestinal tract, urogenital system and metabolism is the principal reason for the increased popularity. With 13 species of pig, however, biomedical research has been largely restricted to the domestic pig derived from the Eurasian wild boar and the warthog. Smaller strains have increasingly come to prominence in non-clinical testing and are widely employed as non-rodent alternatives for regulatory-driven safety programs. For pharmaceutical compounds whose route is dermal in man, there is a growing realization that the minipig is a valid model. Pig skin has been shown to be anatomically, physiologically, biochemically and immunologically similar to human skin. Although the use of the minipig in the development of dermal products is well accepted by regulators, its suitability for a wide range of other study types has been somewhat overlooked although in many ways it is more representative of man than other species. The minipig is now recognized as a suitable non-rodent species for efficacy and safety studies for many types of pharmaceutical products, and are suitable experimental models using many of the commonly applied techniques used in other species. They have also been used as alternative non-rodent species for embryofetal studies.

CE 2 INTRODUCTION TO PATHOLOGY FOR TOXICOLOGISTS AND STUDY DIRECTORS.

L. M. Fomby[1] and P. Haley[2]. [1]*Battelle, Columbus, OH and* [2]*Incyte Corp, Wilmington, DE.*

Today, even with the promise of 'omic' technologies, pathology plays a critical role in the evaluation of chemicals and drugs. Toxicological pathology uses specialized terms and methods and can generates complex interpretative problems. Effective communication between the toxicologist and the study pathologists will allow the toxicologist to better integrate pathology data into their reports and optimize the scientific content of the study. In this basic course, experienced toxicological pathologists will introduce important concepts in pathology to toxicologists as study directors in order to help the study director appreciate the role of pathology in toxicological studies. The course will begin with a discussion of basic concepts and the role of the toxicological pathologist followed by a discussion of global regulations as they relate to pathology endpoints. Later lectures will discuss the lesions and interpretation of pathology data in non-oncogenicity studies and oncogenicity studies. By the end of the session, the study director should have a better understanding of pathology and a basic background that will allow for effective communication with a study pathologist.

CE 3 STEM CELLS AND THEIR MULTI-POTENTIAL USES AND POTENTIAL DANGERS.

D. Lawrence[1] and M. J. Pallardy[2]. [1]*Wadsworth Center, Albany, NY and* [2]*University of Paris, Chatenay Malabry, France.*

Stem cells can theoretically divide without limit to replenish cells and each daughter cell has the potential to remain a stem cell or develop into a fully functional differentiated cell. Therefore, stem cells have the potential to develop into many cell types that could benefit the health status of many individuals but the types of cells that could differentiate from stem cells must be considered in that certain growth and differentiation factors could give rise to conditions that could be harmful including development of a cancer stem cell. Additionally, environmental factors can skew the developmental patterns of stem cells leading to harmful deficiencies or expansions of certain types of progenitors. This course is designed as a basic level course to provide an overview of the field of stem cell biology, touch upon the differences of adult versus embryonic stem cells and provide a foundation for understanding the implications of the use of stem cells in toxicological research. Presentations will cover in vitro and in vivo proliferation and differentiation of stem cells and the influences of toxicants on these processes. Quantification of normal and aberrant immunophenotypic changes with development, mechanisms that influence proliferation, differentiation, and functional changes, and in vivo control of progenitors in their developmental niche will be discussed. The effect of environmental toxicants on a single molecular pathway controlling cell signaling and development of the CNS will be presented. Finally, the challenges in pharmacologically regulated cell therapy including the ability to regulate the fate of engineered cells will be deliberated.

CE 4 DOSE-RESPONSE MODELING FOR OCCUPATIONAL AND ENVIRONMENTAL RISK ASSESSMENT.

D. G. Dolan[1] and A. Maier[2]. [1]*Amgen, Inc., Thousand Oaks, CA and* [2]*Toxicology Excellence for Risk Assessment, Cincinnati, OH.*

The development non-cancer exposure guidance values - whether for environmental or occupational sources of exposure - is rooted in the 'critical adverse effect' concept. Thus, derivation of such guidance values requires understanding the methods and approaches for estimating thresholds for the onset of adverse effects. This course will describe the history of the development of methods to develop 'safe' exposure limits using adverse effect level estimation. The lecture will focus on current methods and issues for using dose-response modeling for developing potency and point of departure estimates for limit-setting. Each lecture will include presentations followed by hands-on application of key elements from the lecture as applied to model toxicological datasets. The participants are expected to obtain sufficient knowledge to estimate critical effect levels for risk assessment using a variety of modeling techniques and addressing key considerations of current interest.

CE 5 THE USE OF TRANSGENIC ANIMAL TECHNOLOGY IN TOXICOLOGICAL RESEARCH.

D. K. Ness[1] and R. Forster[2]. [1]*Elan Pharmaceuticals, Inc., South San Francisco, CA and* [2]*CIT, Evreux, France.*

The ability to direct genetic changes at the molecular level in vivo has resulted in a revolution in biology. Nowhere has this been more apparent than in the production of transgenic animals. A host of techniques has been used to effect change in gene expression and develop new toxicological testing paradigms. Genetically modified animals are commonly produced and often yield important information relevant to safety/toxicological assessment. This session will help guide the toxicologist in the use and interpretation of data derived from transgenic models. This course will introduce this topic and review the history of genetic engineering technologies leading to the development of loss-of-function, gain-of-function modeling technologies across mammalian platforms.

CE 6 PROCESS BASED APPROACHES TO MODULATING GENE AND PROTEIN EXPRESSION *IN VIVO* AND *IN VITRO*.

R. Pollenz[1] and R. Tanguay[2]. [1]*University of South Florida, Tampa, FL and* [2]*Oregon State University, Corvallis, OR.*

The mechanistic analysis of cellular responses to xenobiotics requires the ability to modulate important genes involved in specific pathways. Such genes include those that encode receptors that associate with xenobiotics as well as the enzymes involved in xenobiotic metabolism. The ability to modulate these genes and proteins in vitro and in vivo has become accessible to more laboratories with the refinement of techniques such RNA interference (RNAi), viral gene delivery, morpholino-mediated gene knock down and targeted gene disruption. However, the ability to utilize these techniques and generate reproducible results requires a detailed understanding of the advantages and applications of each procedure. Thus, the goal of this course is to provide the investigator with an overview of experimental design and the use of proper controls for four cutting edge techniques. The first talk will focus on experimental design and analysis of RNAi to reduce endogenous target proteins in culture cells with emphasis on controls and endpoint analysis. The second presentation will move to the zebrafish model system and discuss the use of morpholino-mediated gene knock down to reduce the expression of specific proteins in embryos. The third presentation will discuss gene delivery utilizing the adenovirus system for reduction of gene expression in mice. The forth presentation will detail the use or transgenic approaches in mouse models to modulate the expression of specific target genes or knock-in genes from other species. This course should be of broad interest to laboratories considering a mechanistic approach to understanding signal transduction pathways, gene expression and protein-protein interactions as well as those currently investigating these endpoints.

CE 7 BASIC EMBRYOLOGY AND DEVELOPMENTAL TOXICOLOGY.

L. Dostal[1] and J. M. Rogers[2]. [1]*Private Consultant, Ann Arbor, MI and* [2]*U.S. EPA, Research Triangle Park, NC.*

Embryonic and fetal prenatal development in mammalian species is a complex process which is sensitive to the effects of maternal and environmental factors. The timing of development of major organ systems varies between humans and various

test species used in assessment of developmental toxicity of xenobiotics. This basic course will cover normal development from fertilization through early stages of implantation and embryogenesis, through development of the major organ systems (cardiovascular, central nervous system, craniofacial, skeletal, urogenital). The effects of known human and animal teratogens on fetal development will be presented as examples, including etiologies of abnormal development. Current requirements and study designs for developmental toxicology studies required for new chemicals and new drugs will be covered in the context of the biological concepts of organ systems described in the initial lectures.

CE 8 NANOTOXICOLOGY: THE SCIENCE OF DEVELOPING A SAFE TECHNOLOGY.

C. M. Sayes[1] and J. Teeguarden[2]. [1]*DuPont Haskell Labs, Newark, DE and* [2]*Pacific Northwest National Laboratory, Richland, WA.*

The objective of this course is to prepare toxicologists and risk assessors to meet the rapidly growing need to understand and evaluate the risks that engineered nanomaterials may pose to human health. Toxicological and risk assessment of nanomaterials requires an understanding of the unique differences between these 'new' materials and their chemical and large-particle predecessors. Few currently have the multidisciplinary understanding (toxicology, nanoscience, applied physics, dosimetry) to effectively approach the problem of risk and safety assessment of these materials. This basic course will provide an overview of the state of the science of the field of nanotoxicology and develop the scientific basis for evaluating and characterizing exposure, toxicity and the risks associated with engineered nanomaterials. Nanoscience and nanomaterials will be described with a focus on how they may be different than chemicals and previous studied particulates. A summary of current evidence regarding important routes of exposure and materials humans are exposed to will be presented. Current methods for, and challenges to toxicological testing of nanomaterials will be covered with specific examples relating the toxicity of nanoscale TiO2 to more well characterized particulates (e.g. crystalline silica). Guidelines for testing have been proposed by several groups and will be summarized. Finally, the dosimetric basis for extrapolating the results of toxicology studies to humans will then be developed and presented. This course promotes the application of best scientific practices in the toxicological evaluation and risk assessment of this rapidly growing family of new materials.

CE 9 CLINICAL DOSE SETTING FOR BIOTHERAPEUTICS.

J. D. Green[1] and S. M. Heidel[2]. [1]*Biogen Idec, Inc., Cambridge, MA and* [2]*Eli Lilly and Company, Indianapolis, IN.*

Preclinical development programs that are designed to support the safe clinical use of biopharmaceuticals have considerations that are very different from programs designed to support the development of small molecule drugs. The International Conference of Harmonization ICH S6 document provides guidance regarding scientific and regulatory consensus for key preclinical study design elements. Since the adoption of ICH S6 considerable experience has been developed across a wide variety of biological drug and antibody constructs. This experience has indicated that if ICH S6 guidance is followed and expert assessment of data sets is provided, the safety considerations for first in human and later stage clinical trials has been assured. However, the recent experience involving the humanized monoclonal antibody TGN1412 has raised questions regarding the adequacy of current nonclinical approaches. This session will review the following current and proposed regulatory guidance documents that determine safe starting doses, basic and advanced concepts in PK and PD that support preclinical and clinical dosimetry, basic and advanced concepts of toxicology that are involved in the assessment of safety of biotherapeutics and the current approaches will be illustrated by several case studies across a wide range of product classes. The course attendee will learn key concepts and design considerations for successful preclinical programs that support the initiation of safe human clinical trials.

CE 10 USE OF DATA FOR DEVELOPMENT OF UNCERTAINTY FACTORS IN NON-CANCER RISK ASSESSMENT.

J. Lipscomb[1] and L. T. Haber[2]. [1]*U.S. EPA, Cincinnati, OH and* [2]*Toxicology Excellence for Risk Assessment, Cincinnati, OH.*

Promoting clarity and objectivity is a primary concern when advancing the technical basis for human health risk assessment. Default values for uncertainty factors are intended to serve as place-holders, to be replaced when relevant data become available. The default values for the uncertainty factors for inter and intraspecies extrapolation were established before many toxicologists were born, and methods for incorporation of chemical-specific or categorical data continue to evolve. To understand the rationale for these uncertainty factors and methods for their replacement, this course will present a continuum of approaches to develop non-default values for uncertainty factors, culminating in the application of advanced toxicokinetic methods to quantify differences in internal dosimetry. The course will describe the history of the development of default values for uncertainty factors, including their recent subdivision into toxicokinetic and toxicodynamic components; methods that make use of generally-applicable species differences in anatomy and physiology and allometric scaling; specific instruction in the Chemical Specific Adjustment Factor (CSAF) methodology developed by the International Programme on Chemical Safety (IPCS); and the application of physiologically based pharmacokinetic (PBPK) modeling to develop quantitative data for use in replacing default assumptions for inter and intraspecies differences in tissue dosimetry. The concluding lecture will present a series of examples to illustrate how the various approaches introduced in the course can be applied to derive non-default uncertainty factors for environmental contaminants and pharmaceutical compounds.

CE 11 ESSENTIAL INFORMATICS FOR TOXICOLOGISTS: KNOWLEDGE MANAGEMENT END TO END.

W. B. Mattes[1] and L. D. Burgoon[2]. [1]*The Critical Path Institute, Rockville, MD and* [2]*Toxicogenomic Informatics and Solutions, LLC, Lansing, MI.*

The combination of the Internet, automated data acquisition, and genomic information has transformed the role of the computer in the modern scientist's life. A familiarity with word processing and simple spreadsheets is simply not adequate preparation for dealing with large datasets such as those generated by large toxicology studies, toxicogenomics or high-throughput screens. Increasingly, the software tools used to deal with such data require an understanding of basic concepts in computer science, database design, bioinformatics and statistics. This 'basic' level course hopes to provide the beginnings of such an understanding. Thus the first lecture will cover some of the essential concepts of operating systems, file and data concepts and programming concepts. This will be followed by a talk discussing the essentials of database design and use, contrasting flat-file and relational databases. A third lecture will provide an overview of the information necessary so that data may be integrated, e.g. pathology with genomics. The final lecture will cover concepts of visual analysis of large data sets, and contrast some of the various approaches used. Hopefully after this course the student will be conversant in informatics to the level of effectively interacting with computer scientists, as well as collecting and manipulating datasets with reasonable skill.

CE 12 EPIDEMIOLOGY FOR TOXICOLOGISTS: INTRODUCTION.

R. A. Parent[1] and G. Olsen[2]. [1]*Consultox Ltd., Damariscotta, ME and* [2]*3M Corporation, St. Paul, MN.*

Over the course of the last 50 years, epidemiology has gained respect as an established scientific discipline. Whereas epidemiologic results were once greeted with scorn and skepticism they are now accorded some degree of respect and in some instances perhaps too much respect. This course will begin by providing an overview of the terminology used to describe the techniques used to measure and quantify disease in populations. Toxicologist will become familiar the with the various study designs used in epidemiologic research to generate and evaluate hypotheses regarding disease occurrence. Next, the distinction between experiments and observational studies will be made with an overview on epidemiologic study designs, and bias in epidemiologic data. We will cover the types of observational study designs characterized according to whether the unit of observation is a group, the individual, or some hybrid of the two. A summary will provide the audience with some general guidelines regarding the tendency of the different types of epidemiologic studies to provide unbiased results.

S 13 METALS, MICROGLIA, AND NEUROINFLAMMATION.

D. Lawrence. *Wadsworth Center, Albany, NY.*

The major immune cell that permanently resides within the brain parenchyma is the microglial cell. Current consensus suggests that microglia are derived from the hematopoietic monocyte lineage and that blood monocytes differentiate into perivascular macrophages which develop into microglia as they traffic into multiple brain regions. Another hematopoietic cell residing in the brain is the mast cell. Most other immune cells only transiently migrate through the brain. A major question that continues to be debated is whether neurotoxic metals directly activated immune cells within the brain that subsequently induce neuronal damage or whether metal toxicants induce neuronal damage, which subsequently activates the

immune cells (mainly microglia) leading to exacerbated neuronal damage. An alternative hypothesis is that microglia are neuroprotective but metals cause loss of microglial protective factors because of microglial degeneration and/or elevated production of potential cytotoxicants. Numerous cytokines have been implicated in the neuroinflammation and neurodegeneration, but their cellular sources have not been fully delineated. The various neuroimmune mechanisms posited to regulate neuroinflammation, neuroprotection, and neuronal degeneration will be discussed.

S 14 ROLE OF MICÏROGLIA IN METAL NEUROTOXICITY.

M. Aschner. *Pediatrics, Vanderbilt University Medical Center, Nashville, TN.*

Microglia respond early to neurotoxic insults. Increased evidence suggests that activation of these cells contributes to neuronal damage in multiple neurodegenerative diseases, including Parkinson's disease and Alzheimer's disease. Activation of microglia may ensue as a result of (1) recognition of pro-inflammatory stimuli, such as lipopolysaccharide (LPS) or (2) in response to neuronal damage (reactive microgliosis), which is then toxic to neighboring neurons, resulting in a perpetuating cycle of neuronal demise. Microglial activation can also enhance ongoing neurodegeneration. For example, microglia-derived LPS synergistically enhances both the activation as well as the neurotoxic effects of rotenone and MPTP, suggesting amplification of neuroinflammation processes and the associated neurotoxicity by activated microglia. A common mechanism invoked in mediating microglia-induced neurotoxicity is via release of reactive oxygen species (ROS). The primary and most common pathway by which diverse toxin signals are transduced into ROS production in microglia is via pattern recognition receptors (PRRs). Stimulation of NADPH oxidase activity appears to be the predominant mechanism by which microglia generate ROS. NADPH oxidase is also implicated as a mechanism of proinflammatory signaling in microglia. This talk will address the role of microglia in linking the environment and disease, using several environmental agents as examples. Major objectives of the talk are (1) to discuss the propensity of microglia to accumulate toxins, such as methylmercury (MeHg); (2) to discuss mechanisms associated with microglial activation by meHg and other xenobiotics; and (3) to enumerate common downstream effects associated with microglial activation and neurotoxicity.

S 15 POTENTIAL ROLE OF MICROGLIA IN MANGANESE NEUROTOXICITY.

N. M. Filipov, P. L. Crittenden and S. Lee. *CEHS, Basic Sciences, Mississippi State University, Mississippi State, MS.*

Proinflammatory cytokines and other inflammatory mediators, when produced in substantial amounts, may be involved in neurodegenerative diseases such as Parkinsons Disease (PD). Microglia, the main producers of proinflammatory cytokines in the brain, are not uniformly distributed and are concentrated in the basal ganglia, an area of the brain that is also a target for excessive manganese (Mn) exposure. This presentation will (*i*) describe our findings that Mn enhances the production of lipopolysaccharide (LPS)-induced proinflammatory cytokines such as IL-1β, IL-6, and TNF-α by microglial cell lines and microglia-rich brain slices and (*ii*) present new data pertaining to the mechanism(s) Mn uses to enhance the production of proinflammatory cytokines by activated microglia. More specifically, in series of studies, we have determined that the increased proinflammatory cytokine production by activated microglia exposed to Mn is associated with persistent activation of a key intracellular kinase, p38. Moreover, the prolonged p38 activation caused by Mn exposure appears to be caused by an inhibition of a key phosphatase. In addition, a transcription factor that is potentially involved in proinflammatory cytokine production is markedly increased by Mn in LPS-activated microglia. Increased production of proinflammatory cytokines by microglial cells may cause damage to neighboring neurons. Thus, determining the mechanism(s) of Mn-enhanced proinflammatory cytokine production will contribute to our understanding of Mn neurotoxicity and similar neuropathologies.

S 16 LEAD AND MERCURY INDUCED NEUROIMMUNE CHANGES IN THE BRAIN.

D. Lawrence[1], J. Kasten-Jolly[1], D. Gao[1], T. Mondal[1] and C. Hudson[2].
[1]*Wadsworth Center, Albany, NY and* [2]*SUNY Upstate Medical University, Syracuse, NY.*

Glia activity is modified by developmental lead (Pb) or mercury (Hg) exposure, and these exposures altered behaviors in adults. Exposure via the drinking water to the dam continued from gestational day 8 until weaning at postnatal day (PND) 21. At PND 21, some pups were separated by gender and brains were assayed for mRNA and protein levels for cytokines and immunoregulatory factors. Some mice per litter also were aged an additional 6 wk without metal in their drinking water and assayed for behavioral changes. The two major immune cells residing in brain parenchyma (microglia and mast cells) were specifically assessed. Metal effects on astrocytes also were measured with regard to GFAP expression. Both the Hg and Pb exposure differentially affected the neuroimmune profiles as well as the behaviors of male and female mice. In general, both Pb and Hg appear to enhance processes associated with neuroinflammation. Cytokines that currently appear to be intimately involved in the responses to the metal exposures are IL-6, IL-18, and TGF-beta. Potential mechanisms implicated in the elicited processes will be discussed.

S 17 MICROGLIAL ACTIVATION OR MICROGLIAL DEGENERATION?

W. J. Streit. *Neuroscience, University of Florida, Gainesville, FL.* Sponsor: D. Lawrence.

Neurotoxic metals are thought of as causing injury primarily to neurons, followed by glial activation secondarily. Much interest has been focused on the activation of microglia because activated microglia are often seen as potentially dangerous immune effector cells believed to exacerbate the initial toxic insult. Activated microglia have also been linked causally to the development of neurodegenerative diseases, such as Alzheimer's and Lou Gehrig's disease. On the other hand, there is reason to believe that activated microglia are neuroprotective and important for rescuing injured neurons. Based on this idea, we have reexamined microglial activation in neurodegenerative disease states in both human and rodent brain, and our findings strongly suggest that many ostensibly activated microglia are, in fact, degenerating microglial cells. These observations have led us to propose a novel 'microglial dysfunction hypothesis' regarding the role of activated microglia in the pathogenesis of neurodegeneration. The hypothesis states that it is the loss of microglia and/or microglia neuroprotective functions that triggers neurodegeneration. This view represents a paradigm shift offering a revised perspective on the functional significance of activated microglia. It should prompt studies to investigate if traditional neurotoxins might also have microgliotoxic properties.

S 18 MOLECULAR BASIS FOR SUSCEPTIBILITY TO CHEMICAL TOXICITY AND DISEASE.

R. Hines. *Medical College of Wisconsin & Children's Research Institute, Milwaukee, WI.*

The completion of the Human Genome Project in 2003, and more recently, the first phase of the International HapMap project, have provided valuable tools which we are still learning how to use effectively in our goal to improve human health and prevent disease. For example, the cost-effective and rigorous ability to perform whole genome association studies for complex diseases has only been achieved within this past year. Although exciting and of clear utility in identifying multiple genetic loci whose variability will contribute to disease risk, such association studies still must be followed-up with more detailed analyses to understand the causative variants that contribute to differential susceptibility. Further, there now is widespread acceptance that most if not all complex disease arises because of an interaction between our genome and the environment. Thus, a careful assessment and definition of phenotype, including environmental exposure, is critical. We also now have clear examples wherein epigenetic changes caused by environmental factors, including transgenerational epigenetics changes, can also have a significant impact on disease risk. This symposium will provide state-of-the-art updates on our understanding of the molecular basis for individual differences in susceptibility to chemical toxicity and disease. Topics to be covered include how regulatory polymorphisms resulting in variation in gene expression can impact risk, chemical-induced epigenetic changes and their impact on susceptibility, the use of genetic variation to predict risk and efficacy during drug development and the use of pathway analyses to understand complex disease risk using Parkinson's disease as an example. Thus, this symposium will span from fundamental, mechanistic studies, to translational investigations and clinical applications, demonstrating the strengths of an interdisciplinary approach to environmental health issues.

S 19 REGULATORY POLYMORPHISMS AND THEIR IMPACT ON SUSCEPTIBILITY.

R. N. Hines. *Pediatrics, Medical College of Wisconsin & Children's Research Institute, Milwaukee, WI.*

CYP2C9 accounts for approximately 20% of the total cytochrome P450 content in adult human liver and is responsible for the metabolism of approximately 16% of clinically used drugs, as well as numerous environmental toxicants. Although variation within the *CYP2C9* structural gene has been extensively characterized, the identified variants fail to fully explain interindividual *CYP2C9* expression differences. To address this problem, 31 SNPs were identified in 10 kbp of *CYP2C9* upstream information using the 24 DNA sample set from the Coriell PDR. Individual

SNP frequencies were determined in 193 DNA samples obtained from Hispanic Americans (Mexican descent) and used to infer common haplotypes using PHASE 2.1. Nine common variant haplotypes were inferred, two of which included the *CYP2C9*2* and two the *CYP2C9*3* causative SNPs. The variant haplotypes were introduced into a *CYP2C9*/luciferase reporter construct and their impact on promoter activity evaluated by transient expression in HepG2 cells. Relative to the reference, 3 haplotypes exhibited a 65% decrease in constitutive promoter activity while 2 exhibited a 50% decrease or a 40% increase in PXR-mediated, rifampicin-induced promoter activity.

The flavin-containing monooxygenases (FMOs) also are important for xenobiotic metabolism. FMO3 is expressed at levels comparable to CYP2C9 in the adult liver and is the predominant FMO enzyme in this tissue. However, significant interindividual variation is observed. Using a similar approach as that used for *CYP2C9*, *FMO3* population-specific SNP frequencies were determined and haplotypes were inferred. Of the 7 common promoter region haplotypes identified, one resulted in an 8-fold increase in *FMO3* promoter activity, while two exhibited a near complete loss of activity.

Although the in vivo significance of both the *CYP2C9* and *FMO3* regulatory polymorphisms has yet to be determined, their ability to significantly alter their respective promoter activities would suggest such genetic variants will likely contribute to a risk phenotype for xenobiotics dependent on these enzymes for their disposition.

S 20 EPIGENETICS AND ITS IMPORTANCE IN TOXICOLOGY.

R. L. Jirtle. *Radiation Oncology, Duke University Medical Center, Durham, NC.*

Human epidemiological and animal experimental data indicate that the risk of developing adult-onset diseases, such as asthma, diabetes, obesity, and cancer, is influenced by persistent adaptations to prenatal and early postnatal exposure to environmental factors. Moreover, the link between what we are exposed to *in utero* and disease formation in adulthood appears to involve epigenetic modifications like DNA methylation at metastable epiallele and imprinted gene loci.

Genomic imprinting is an epigenetic form of gene regulation that results in monoallelic, parent-of-origin dependent gene expression. The functional haploid state of imprinted genes makes them susceptibility loci for diseases since a single genetic or epigenetic mutation can alter their function. We have recently developed a computer-learning algorithm that identified 600 imprinted-candidate genes in the mouse. Interestingly, humans are predicted to not only contain fewer imprinted genes, but the repertoire is strongly species dependent (unpublished results). By mapping the human imprinted-gene candidates onto the landscape of disease risk defined by linkage analysis, we are now poised to determine the importance of imprinting in the etiology of complex human diseases.

Genes with metastable epialleles have highly variable expression because of stochastic allelic changes in the epigenome rather than mutations in the genome. The viable yellow agouti (A^{vy}) mouse harbors a metastable *Agouti* gene because of an insertion of a transposable element. We have used the A^{vy} mouse to investigate the importance of nutrition in determining the susceptibility of offspring to adult diseases. We have shown that maternal dietary supplementation during pregnancy, with either methyl donors (i.e. folic acid, vitamin B_{12}, choline and betaine) or genistein, decreases adult disease incidence in the offspring by increasing DNA methylation at the A^{vy} locus. Moreover, these nutritional supplements can block CpG hypomethylation caused by the endocrine disruptor, bisphenol A. (Supported by NIH grants ES13053, ES08823, ES015165 and T32-ES07031, and DOE grant DE-FG02-05ER64101)

S 21 THE USE OF GENETIC VARIATION TO PREDICT RISK AND EFFICACY DURING DRUG DEVELOPMENT.

A. D. Roses. *Joseph and Kathleen Bryan Alzheimer's Disease Research Center, Durham, NC.* Sponsor: R. Hines.

Safety and efficacy pharmacogenetics can be applied successfully to the drug discovery and development pipeline at multiple phases. Several examples will be discussed. We have conducted screening using high throughput SNP associations with Alzheimer's disease, testing more than 1,800 candidate targets with approximately 7,000 SNPs in an attempt to identify novel drug-targets. A Phase IIB clinical trial for Alzheimer's disease will be described that exemplifies the major pipeline decision between program attrition and further clinical development. In this case, there was no significant improvement in 511 intention-to-treat patients but, applying a confirmed prognostic biomarker (*APOE4*) to segment the clinical trial population, all three doses of rosiglitazone demonstrated improvement in patients who did not carry the *APOE4* allele. The data for the *APOE4* carriers demonstrated no significant improvement but suggested that there may be a need for higher doses. Thus, a development program that would have been terminated progressed to Phase III registration trials based on the results of prospective efficacy pharmacogenetic analyses. The implications of using *APOE* genotype as a biomarker to predict efficacy and possibly dose, as well as supporting the basic neurobiology and pharmacology that provided the original target validation, will be discussed. Data will be presented that support a slow neurotoxic effect of a specific fragment of apoE protein on mitochondria and the use of rosiglitazone to increase mitochondrial biogenesis and improve glucose utilization. In summary, pharmacogenetics is currently being used across the pipeline to prevent attrition and to create safer and more effective medicines.

S 22 A GENOMIC PATHWAY APPROACH TO COMPLEX DISEASES: PROMISE, PITFALLS, AND REMEDIES.

D. Maraganore. *Mayo Clinic College of Medicine, Rochester, MN.* Sponsor: R. Hines.

We recently described a genomic pathway approach to study complex diseases [Lesnick et al., 2007]. We illustrated this approach using the example of the axon guidance pathway and Parkinson's disease (PD), and using available data from a whole-genome association study of PD [Maraganore et al., 2005]. We produced single nucleotide polymorphism (SNP) models that were highly predictive of PD susceptibility (odds ratio = 90.8, $p = 4.64 \times 10{-}38$), survival free of PD (hazards ratio = 19.0, $p = 5.43 \times 10{-}48$), and PD age at onset ($R2 = 0.68$, $p = 1.68 \times 10{-}51$). We were able to validate our findings for the axon guidance pathway and PD using available SNP data from a second whole-genome association study of PD and also using available probe set data from a whole-genome expression profiling study of PD [Lesnick et al., 2007]. Our findings for the axon guidance pathway and PD have biological plausibility. With respect to a possible brain wiring mechanism, they support the fetal basis for adult diseases (FeBAD) hypothesis (consistent also with animal models of neurodevelopmental toxicity and PD). With respect to a possible axonal maintenance and repair mechanism, they support the role of cumulative toxicity with aging. A genomic pathway approach might similarly provide mechanistic insights into the causes of other diseases of relevance to toxicology. However, we acknowledge that findings for genomic pathways and complex diseases may prove difficult to replicate across diverse populations, particularly using SNP markers. Furthermore, traditional experimental models may not embrace the complexity of the genomic pathway findings observed for human subjects. In light of such pitfalls, we will discuss additional approaches that can be employed to validate findings for genomic pathway and complex diseases, again using the example of the axon guidance pathway and PD, with hopes of fulfilling the promise of the genomic pathways approach.

S 23 NEW DEVELOPMENTS IN LIVER TUMOR BIOLOGY.

R. Roberts[1] and Y. Dragan[2]. [1]*R&D Safety Assessment, AstraZeneca, Macclesfield, United Kingdom and* [2]*R&D Safety Assessment, AstraZeneca, Wilmington, DE.*

The liver is one of the most widely studied organs in cancer biology since it provides a flexible model for analysis of tumor biology that is amenable to in vitro and in vivo correlative approaches. Because of this, many other fields of cancer biology have been informed by data emerging from studies of the liver. Over the last decade, the inter-related roles between the pathways of apoptosis, cell survival and cell proliferation and their relevance to toxicology have been more rigorously described. Key to this is the interplay between ligand-activated transcription factors such as the peroxisome proliferators activated receptor α (PPARα) and the constitutively active/androstane receptor (CAR) and the networks that initiate and execute apoptosis. Against this background of knowledge, several new findings are particularly resonant. Specifically, increasing evidence suggests a pivotal role for epigenetic modification of DNA and its impact on survival and proliferation. Also of great relevance is the role played by the wnt-signalling pathway in cell signalling and tumorigenesis. Another recent and informative approach involves the use of comparative genomics of human and model organism hepatocellular carcinoma to identify aberrant phenotypes, reflecting molecular pathways that are evolutionarily conserved. This is further illustrated by use of a systems toxicology approach to analysis of liver tumors. This symposium brings together leading experts in human cancer biology and liver toxicology to provoke cross-fertilization of ideas and will be of interest both to non-experts looking to gain a greater understanding of tumor biology and those with a specific interest in the liver and mechanisms of carcinogenesis.

S 24 THE ROLE OF CELL SURVIVAL SIGNALLING IN NORMAL AND TUMOR CELL BIOLOGY: LESSONS FROM LIVER.

R. Roberts. *Safety Assessment, AstraZeneca, Macclesfield, United Kingdom.*

There are multiple pathways implicated in regulating cell survival and apoptosis, and much of this has been elucidated in the liver as well as in other systems. Apoptosis can be induced via two main pathways, the stress/damage pathway and

the death receptor pathway. In general, stresses such as DNA damage, oxidative stress and chemical stress signal via the mitochondria to activate caspase 9 which in turn feeds into Caspase 3. In contrast, death ligands such as FasL and TNFa activate an extensive family of specific cell surface receptors and cascade through to caspase 3 activation via the Death Inducing Signalling Complex (DISC). These pathways of induced cell death are highly relevant to our understanding of tumor cell biology and will be described in order to set the scene for the subsequent presentations on signalling, epigenetics, and carcinogenesis. Overall, key developments in elucidating cross-talk between survival and proliferation networks is highly relevant to our understanding of both normal and tumor cell biology.

S 25 THE ROLE OF THE WNT PATHWAY AND β-CATENIN IN NORMAL AND TUMOR CELL BIOLOGY.

S. A. Aaronson. *Department of Oncological Sciences, Mount Sinai School of Medicine, New York, NY.* Sponsor: R. Roberts.

The evolution of multicellular organisms requires intercellular communication for processes including embryonic development, tissue differentiation, and systemic responses to wounds and infections. These complex signaling networks are in large part mediated by growth factors, cytokines and hormones. The Wnt family of highly conserved, secreted signaling molecules is essential in embryonic induction, cell polarity generation, and cell fate specification. Mutations in components, which constitutively activate Wnt canonical signaling, have been identified in several tumor types. Wnts bind to two co-receptors, the Frizzled-type seven-transmembrane-domain receptor and the low-density receptor related protein (LRP) 5/6. These interactions cause β-catenin stabilization through inhibition of its phosphorylation by glycogen synthase kinase 3β (GSK3β). Phosphorylation of β-catenin normally targets it for proteosome degradation, and inhibition of its phosphorylation leads to stabilization and translocation to the nucleus, where it forms a complex with a family of high mobility group (HMG)-like transcription factors including leukocyte enhancer factor-1 (LEF-1) and T cell factors (TCF), and activates the transcription of target genes. Several naturally occurring, secreted antagonists of Wnt signaling have been identified, and these act either to competitively inhibit Wnt/receptor interactions or by specifically interfering with signaling by LRP5/6. It is well established that genetic aberrations leading either to loss of function of APC or activating mutations in β-catenin, which impair its proteosome degradation, result in increased levels of uncomplexed β-catenin and constitutive activation of Wnt canonical signaling in human malignancies including hepatocellular carcinoma. Current efforts are focused on how activation of Wnt canonical signaling plays such an important role in such a wide array of human tumors

S 26 ORPHAN NUCLEAR RECEPTOR CONSTITUTIVE ACTIVE/ANDROSTANE RECEPTOR (CAR)-MEDIATED ALTERATIONS IN DNA METHYLATION DURING PHENOBARBITAL (PB) PROMOTION OF LIVER TUMORIGENESIS.

J. I. Goodman[1] and J. M. Phillips[2]. [1]*Pharmacology and Toxicology, Michigan State University, East Lansing, MI and* [2]*Biochemistry and Mollecular Biology, Michigan State University, East Lansing, MI.*

Altered DNA methylation is an epigenetic mechanism that plays a key role in the carcinogenesis process, and the nongenotoxic rodent hepatocarcinogen phenobarbital (PB) alters the methylation status of DNA in mouse liver. The constitutive active/androstane nuclear receptor (CAR) mediates half of the PB-induced hepatic gene expression changes and it is essential for liver tumor promotion in PB-treated mice. Methylation-sensitive restriction digestion, arbitrarily primed PCR, and capillary electrophoresis were utilized to detect PB-induced regions of altered DNA methylation (RAMs) in CAR wildtype (WT) mice that are sensitive to promotion by PB and resistant CAR knockout (KO) mice, both on a C3H/He background. The CAR WT mice developed preneoplastic lesions after 23 wk of PB treatment (precancerous) and liver tumors after 32 wk, while the CAR KO mice did not develop tumors (Yamamoto et al., 2004). Our goal is to discern those RAMs that are playing important roles in tumor formation by comparing the RAMs that form in sensitive and resistant groups of mice. Using this novel approach, 42 unique RAMs were identified in the precancerous as compared to the CAR KO, 23 wk PB-treated tissue. Of these 42 RAMs, 14 carried forward to the tumor tissue, and additionally, 104 total unique RAMs were observed in the tumor tissue. These results indicate that unique RAMs occur in the sensitive CAR WT mice and a subset of these is seen in both the precancerous and tumor tissue. Furthermore, 7 of the unique RAMs discerned in the precancerous liver and 13 of those in the tumor tissue were also identified in the livers of B6C3F1 mice treated with a promoting dose of PB for only 4 wk. We hypothesize that the unique RAMs may facilitate the tumorigenesis process, and these data support the view that DNA methylation plays a causative role in PB-induced tumorigenesis. The implications for safety assessment will be discussed.

S 27 GENOMIC DECODING OF HUMAN LIVER CANCER.

S. S. Thorgeirsson. *Laboratory of Experimental Carcinogenesis, NCI, Bethesda, MD.* Sponsor: R. Roberts.

Much is known about both the sequential cellular changes that precede the formation of hepatocellular carcinoma (HCC) and the etiological agents (i.e. HBV, HCV infection, and alcohol) responsible for the majority of HCC. We have investigated the possibility that variations in gene expression of HCC at diagnosis would permit the identification of distinct subclasses of HCC patients with different prognoses.Unsupervised classification methods based solely on gene expression patterns revealed two subclasses of HCC strongly associated with the length of patients' survival. Also, when the classifiers used in a training set to optimized classification of the tumors were applied to the validation set, all the classifiers successfully separated poorer survival patients (cluster A) from longer survival patients (cluster B).
The variability in the prognosis of individuals with HCC may result from activation of different oncogenic pathways during tumorigenesis and/or from a different cell of origin. We have address whether the transcriptional characteristics of HCC can provide insight into the cellular origin of the tumor. We integrated gene expression data from rat fetal hepatoblasts and adult hepatocytes with HCC from human and mouse models. Individuals with HCC who shared a gene expression pattern with fetal hepatoblasts had a poor prognosis. The gene expression program that distinguished this subtype from other types of HCC included markers of hepatic oval cells, suggesting that HCC of this subtype may arise from hepatic progenitor cells. Analyses of gene networks showed that activation of AP-1 transcription factors in this newly identified HCC subtype might have key roles in tumor development.
These findings support the notion that multiple molecular pathways dictate the development and different clinical outcomes of HCC. Therefore, use of gene expression profiling promises to improve molecular classification and prediction of outcomes in HCC. Furthermore, molecular stratification of individuals with HCC into homogeneous subgroups may provide opportunities for the development of new treatment modalities.

S 28 SYSTEMS TOXICOLOGY: APPLICATIONS TO RAT MODELS OF CARCINOGENESIS.

Y. Dragan[1], R. Beger[2], R. Edmondson[2], J. Fuscoe[2], H. Fang[2] and W. Tong[2]. [1]*Safety Assessment-U.S., AstraZeneca, Wilmington, DE and* [2]*Systems Toxicology, NCTR/FDA, Jefferson, AR.*

A number of compounds can cause an increased incidence of liver tumors in rats through mechanisms that may not be applicable in the human. In order to gain a clearer understanding of the mechanisms that underlie liver tumor promotion in the rat, an integrated OMICs analysis was performed on the livers of rats administered selected rodent liver carcinogens including mestranol, phenobarbital or tamoxifen for six months. Microarray analysis was performed on RNA isolated from these livers and 70 genes were changed by any one of the treatments. Proton NMR-based metabonomic analysis was performed and indicated that perturbation of lipids occurred with tamoxifen and phenobarbital administration. Proteomic analyses indicated that 125 proteins were altered in level between the tamoxifen-treated and control liver. This integrated OMIC data analysis produced with ArrayTrack provides important insight into the mechanisms of liver carcinogenesis. Furthermore, the systems toxicology approach is a mecahnism for generating safety biomarkers that can be used to bridge preclinical and clinical datasets. The perturbations of pathways by agents can be compared using a systems toxicology approach.

S 29 INTRODUCTION.

D. S. Barber. *University of Florida, Gainesville, FL.*

Concern is increasing regarding the potential adverse health effects of materials in the nanoscale range, including both ambient air pollutants and manufactured nanoparticles. The extremely high surface area of these materials relative to their mass suggests the potential for interactions with biological molecules, resulting in either inactivation or local concentration of the biological materials. Nanomaterial surface properties, including charge, reactivity, hydrophobicity, and granularity, vary as a function of the source and chemical composition. There is, therefore, potential for some degree of selectivity in the interactions with biological materials, which may influence subsequent responses to the particles. This symposium focuses on recent investigations into these interactions and their biological implications.

S 30 THE ADSORPTION OF BIOLOGICAL MACROMOLECULES BY COMBUSTION-DERIVED NANOPARTICLES.

K. BéruBé[1] and T. Jones[2]. [1]*School of Biosciences, Cardiff University, Cardiff, United Kingdom and* [2]*School of Earth, Ocean and Planetary Sciences, Cardiff University, Cardiff, United Kingdom.* Sponsor: J. Seagrave.

Protein separation by SDS-PAGE can be used to estimate relative molecular mass, to determine the relative abundance of major proteins in a sample, and to determine the distribution of proteins among fractions. For biological purposes, SDS-PAGE can be used to evaluate the adsorptive capacity of biological macromolecules by particulate dusts. The physicochemical properties of dust particles (i.e. size, shape, surface chemistry) plays an important role in their biological activity, in association with biological solutions (e.g. serum proteins and pulmonary surfactant), and hence, their impact of respiratory health. SDS-PAGE of proteins bound to particles can be used to demonstrate whether particles selectively adsorb and retain them. Differential absorption of lung lining fluids by combustion-derived nanoparticles (carbon black, fly ash, diesel particles) may play a role in their pathogenic mechanisms of action in the lung environment.

S 31 USING PROTEOMIC ANALYSIS TO DETERMINE COMPETITIVE BINDING OF PROTEINS TO NANOPARTICLES.

S. C. Wasdo. *University of Florida, Gainesville, FL.* Sponsor: D. Barber.

Proteins readily adsorb to the surface of nanoparticles in a physiological environment, and these proteins can strongly influence the nature and magnitude of a nanoparticle-induced biological response. To more fully understand how various surface characteristics influence protein binding to nanoparticles, it is necessary to investigate the simultaneous and competitive binding of hundreds of proteins. Isotope labeling proteomics techniques, principally ICAT and ITRAQ, have been used to identify and quantify the relative abundance of over 100 adsorbed proteins on various nanoparticles following their exposure to human plasma or serum. Characteristic patterns of protein adsorption were observed in nickel, aluminum, diamond and functionalized gold nanoparticles, including the preferential adsorption of several complement components and other potentially inflammatory factors such as vitronectin. From these data, it is possible to estimate the relative binding affinities of the adsorbed proteins and model the process of competitive protein adsorption in complete physiological fluids.

S 32 SURFACE PROPERTIES CONTROL INTERACTIONS WITH CYTOSKELETAL ELEMENTS.

J. L. McGrath. *Biomedical Engineering, University of Rochester, Rochester, NY.* Sponsor: D. Barber.

We have modified the surface of polystyrene nanoparticles (NPs) with a variety of functional groups and examined the internalization kinetics and processing of these NPs by cultured endothelial cells and fibroblasts. Results reveal several principles that appear to simplify our understanding of NP fate during cellular interactions. First, we show that the capacity of NPs to bind serum proteins directly correlates with the tendency of particles to be internalized by cells. Second, we show that particles that are internalized through endocytosis steadily accumulate in perinuclear stores in a manner that is independent of NP surface chemistry but dependent on cytoskeletal activity. Finally, we show that NPs that emerge free in cytoplasm will interact directly with elements of the cytoskeleton and that these interactions control the diffusive movement of NPs within cells. The extent to which NPS in cytosol bind to either intermediate filaments or actin filaments varies with different surface chemistry.

S 33 MECHANISMS AND IMPLICATIONS OF PARTICLE/CHEMOKINE INTERACTIONS.

J. Seagrave. *Lovelace Respiratory Research Institute, Albuquerque, NM.*

Inflammation induced by inhalation of air pollutant particles has been implicated as a mechanism for the adverse health effects associated with exposure to air pollution. The inflammatory response is associated with upregulation of various pro-inflammatory cytokines and chemokines. Both the neutrophil chemokines human IL-8 and rodent MIP-2 become associated with diesel exhaust particles, and remain active. This presentation will describe the mechanisms for adsorption of these chemokines to DEP and other particles. The biological implications include local concentration of the chemokines and prolonged retention.

S 34 ROLE OF IRON SEQUESTRATION IN HOST RESPONSE TO PARTICLES AND FIBERS.

A. J. Ghio. *Human Studies Division, National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC.*

The host response to particles and fibers includes an increased local expression of iron transport and storage proteins in respiratory epithelial cells which function to sequester iron away from the particle and fiber. Decreased iron complexation by functional groups at the surface of the particle or fiber decreases the oxidative stress. This response is recognized by the presence of ferruginous bodies in a tissue.

W 35 CURRENT PERSPECTIVES ON OCULAR AND SAFETY RISKS OF THERAPEUTICS.

M. D. Aleo and C. A. Somps. *Drug Safety R&D, Pfizer Global R&D, Groton, CT.*

Ophthalmic safety risks can occur from systemically administered therapeutics while systemic safety risks can occur from ophthalmic therapeutics. Such adverse safety effects identified during the research, development, and marketing phase of a new therapeutic agent may drastically limit further progression or marketing depending upon: the nature and severity of the finding, the stage of development, the proposed indication for the new drug, therapeutic index, and relevance of the lesion to humans based on mechanistic elucidation. The goal of this symposium is to present the Yin and Yang of managing ocular safety risks of systemic therapeutics and systemic/ocular safety risks of ophthalmic therapeutics in the pharmaceutical industry and potential methods for managing risks through mechanistic evaluation. Specific emphasis will be placed on the following concepts: 1) unexpected lenticular toxicity associated with therapeutic agents intended for systemic administration along with ways to screen potential back-up candidates and risk assessment strategies, 2) unintended systemic toxicity associated with therapeutic agents intended for ophthalmic administration, 3) newest concepts and challenges associated with the development of antisense, nanotechnology, and biologic-based therapeutics for ophthalmic indications, implantable ophthalmic delivery devices and special formulations for ophthalmic delivery and 4) a perspective on how such cases may be viewed by medical reviewers for regulatory agencies. As a workshop this course will promote interaction with the audience to share experiences in managing ocular/systemic safety risks in a pharmaceutical setting.

W 36 LENTICULAR TOXICITY OF SYSTEMIC THERAPEUTICS IN THE RAT: CASE STUDY AND MECHANISM-BASED SCREENING APPROACHES.

M. D. Aleo. *Drug Safety R&D, Pfizer Global R&D, Groton, CT.*

In the pharmaceutical industry the discovery of ocular toxicity associated with therapeutic agents intended for prolonged systemic administration can lead to compound or program attrition before there is an opportunity to prove clinical efficacy or benefit at sufficient therapeutic margins of safety. Since safety evaluation of therapeutic agents in the rodent is required by regulatory agencies as a nonclinical species for registration of first in human trials, the ability to extrapolate ocular safety risks uncovered during safety testing in rodents is important. A long recognized mechanism of cataract liability in the rodent is related to inhibition of lenticular cholesterol biosynthesis. We have examined this mechanism across a sampling of rodent cataractogens using two distinct mechanistic assays (ex vivo lens cholesterol biosynthesis rate and cholesterol domain formation in model and rat native membranes). Information on the sensitivity and specificity of the assays will be provided. A proposed risk management strategy will be presented for discussion based on the mechanism of toxicity and its relevance to human exposure.

W 37 LENTICULAR TOXICITY OF SYSTEMIC THERAPEUTICS IN THE DOG: CASE STUDY AND MECHANISM-BASED SCREENING APPLICATIONS.

C. J. Somps. *Drug Safety R&D, Pfizer, Groton, CT.*

During drug development, ocular lens toxicity may be observed in one or more preclinical animal models following systemic administration of candidate medicines. Understanding the mechanism(s) by which a systemically delivered compound causes lens toxicity can inform risk management strategies for that particular drug candidate, and may be used to support higher throughput screening strategies for backup compounds or alternative drug development programs. In this session we will review the potential role of membrane ion transport mechanisms in lens toxicity by considering a case study in which systemically delivered drug candidates pro-

duced lens opacities in the dog which correlated with inhibition of potassium currents in lens epithelium. This mechanism was consistent with differences in lens opacity observed across preclinical species, may inform human risk assessment, and provided a path for developing an in vitro, higher throughput tool for screening compounds of similar structure for cataract risk.

W 38 SYSTEMIC CONSIDERATIONS WITH OCULAR PHARMACOLOGIC THERAPIES.

R. B. Bhisitkul. *Department of Ophthalmology, UCSF, Beckman Vision Center, San Francisco, CA.* Sponsor: M. Aleo.

It is not generally well-recognized that severe unintended systemic toxicity can be associated with administration of ophthalmic drugs. For example, ocular administration of chloramphenicol or timolol can be associated with clinical evidence of aplastic anemia or alterations in cardiopulmonary function, respectively. This session will present the latest research regarding the possibility of an increased risk of stroke with Lucentis and off-label use of Avastin. Clinical findings to date suggest that even small amounts of an ophthalmic drug, even a biologic, administered into the eye may have undesirable systemic side-effects in humans.

W 39 NONCLINICAL STUDIES FOR OPHTHALMIC DRUG DEVELOPMENT.

Z. Chen. *U.S. FDA, Silver Spring, MD.* Sponsor: M. Aleo.

This section will focus on nonclinical studies required for ophthalmic drugs. Non clinical evaluation of ophthalmic drug products includes review of preIND, IND, and NDA submissions, and special study protocols. The evaluation is guided by related FDA guidance, and the International Conference for Harmonization (ICH) guidelines. Ophthalmic drug products can be administered topically (as drops applied to the eye), intravitreally, and by several other routes. Regardless of the mode of administration, ocular and systemic toxicity concerns need to be addressed for developing these products. The pharmacokinetics of ophthalmic drugs, including systemic bioavailability and ocular distribution following topical exposure, should be defined early in the development process. As a general rule, ophthalmic drugs should be evaluated in general toxicology, genetic toxicology, reproductive toxicology, and, if applicable, carcinogenicity studies as defined by ICH guidelines. To fully define the toxicity profile of ophthalmic drugs, general toxicology studies should be conducted using both ocular and systemic routes. Ocular toxicity should be assessed in two species. To support human trials, the dosing frequency and drug concentrations in the nonclinical studies should be at least equal and preferably exceed the maximum frequency and concentration of clinical human studies. The assessment of ocular toxicity should include slit lamp biomicroscopy, funduscopy, tonometry, and histopathology of ocular tissues at appropriate times. If the drug binds to melanin, animals with pigmented eyes should be used.

W 40 STRATEGIES FOR NONCLINICAL SAFETY ASSESSMENT OF NOVEL OCULAR PRODUCTS.

D. J. Kornbrust. *Preclinsight, Reno, NV.* Sponsor: M. Aleo.

This session presentation will address general considerations, strategies and regulatory expectations for achieving proper safety assessment of specific types of novel ocular therapeutics, with a primary focus on oligonucleotide-based products (including siRNA programs), as well as other examples of ocular biotechnology-derived therapeutics and novel sustained release technology applications. Attendees should derive a sense for how a rational case-by-case approach to toxicity evaluation and effective collaboration with regulatory agencies can yield the most appropriate nonclinical support for enabling clinical trials.

W 41 THE LLNA, FALSE POSITIVES, FALSE NEGATIVES AND ALTERNATIVE ENDPOINTS.

D. Basketter. *St John's Institute of Dermatology, St Thomas' Hospital, London, United Kingdom.*

The local lymph node assay (LLNA) is being used increasingly in the identification of skin sensitising chemicals for regulatory purposes. In the context of new chemicals legislation (REACH) in Europe, it is the preferred assay, as it is for an increasing number of regulations and regulatory agencies. An important part of the rationale for this is that the LLNA offers a quantitative and objective approach to skin sensitisation testing allied with the important animal welfare benefits. However, as with certain guinea pig sensitisation tests before it, this increasing use also brings experience with an increasingly diverse range of industrial and non-industrial chemicals where the outcome of the assay does not always necessarily meet with the expectations of those conducting it. Sometimes, the result appears to be a false negative, but recently more common has been the suggestion that some chemicals represent false positives. Typically, this situation can arise when the chemistry and guinea pig test history with similar materials suggests possible overestimation of the hazard. Against this background this session aims to review instances where false positive and false negative results have been described and will attempt to reconcile science with expectation. The consequences of confusion surrounding this subject will be considered in relation to efforts to produce valid non-radioactive endpoints for the LLNA as well as in the context of integrating potency measurement and risk assessments into classification and labelling schemes whose aim is to manage potential risks to human health.

W 42 THE LOCAL LYMPH NODE ASSAY: INTRODUCTION, CONDUCT AND PERFORMANCE.

I. Kimber. *Central Toxicology Laboratory, Syngenta, Cheshire, United Kingdom.*

The mouse local lymph node assay (LLNA)is a method for the identification (and characterization) of chemicals with the potential to cause skin sensitization. Activity is measured as a function of the ability of test chemicals to cause an increase in the proliferative activity of of cells within lymph nodes draining the site of topical exposure. In the LLNA skin sensitizing chemicals are defined as those that elicit a 3-fold or greater increase in lymph node cell proliferation (measured by incorporation of radiolabelled thymidine) compared with concurrent vehicle-treated controls. Based upon extensive national and international inter-laboratory evaluations, and detailed comparions with other available methods, the LLNA has been validated as a stand-alone method for the purposes of hazard identification. In the period since formal validation in both the US and Europe the LLNA has found increasing use for assessment of skin sensitizing activity of a wide range of chemicals, and with this accumulating experience has emerged a more detailed understanding of the performance characteristics of the method. In tandem there has been interest in considering the potential utility of alternative end points for the LLNA. The background to these developments will be described and the current status of the assay discussed.

W 43 ALTERNATIVE METHODS AND ENDPOINTS IN "ENHANCED" LOCAL LYMPH NODE ASSAYS AND THE PHOTO-LLNA.

G. L. DeGeorge. *MB Research Laboratories, Spinnerstown, PA.*

The LLNA has evolved greatly from the original concepts of Basketter, Kimber, and Gerberick, recognized as the co-inventors of the assay in the 1990s. Several alternative endpoints and methods have been developed, e.g. Flow Cytometry-based (FC-LLNA), the IMDS test, and the Enhanced-LLNA (E-LLNA), as well as attempts at measuring induction of cytokine levels and release in ex-vivo methods using cultured LNCs. Earlier methods were of varied design and more crude endpoints, ie. "ear weight" and "node weight" for endpoints, and a 4 or 10-day study length in contrast to the current validated standard 6-day protocol. These earlier methods were plagued by false-positives (due to irritancy properties of the test material) and concordance values only in the mid-80% range, at best. Also, the 'pooling' of nodes from all animals in a treatment group did not allow for statistical analyses, including the variance and STDev to assist in identification of 'outlier' data points. The newer methods include quantitative measurements of ear thickness to better detect irritancy; immunophenotyping by flow cytometry to measure cell surface markers of lymphocyte activation; and the separate counting by flow cytometry to better exclude non-lymphocytes, RBCs, and other LN debris. Other factors, including selection of proper vehicle, and range-finding the ideal test doses will be discussed. A summary or timeline of contributions or achievements from Europe, the USA and Japan will also be discussed, along with the latest discoveries, and promising future methods and protocols.

W 44 USE OF THE LOCAL LYMPH NODE ASSAY FOR SENSITIZATION HAZARD IDENTIFICATION: A FIVE-YEARS EXPERIENCE FROM A REGULATOR'S POINT OF VIEW.

A. Oberemm, H. B. Richter-Reichhelm and U. Gundert-Remy. *Safety of Substances and Preparations, Federal Institute for Risk Assessment, Berlin, Germany.* Sponsor: D. Basketter.

Since clinical data are generally sparse, the evaluation of the potential of a substance to cause skin sensitization in humans is mainly based on results prospectively obtained from animal studies. For decades, sensitization testing was performed in

guinea pigs (guinea pig maximization test/ GPMT; exceptionally: Buehler patch test). In 2002, the local lymph node assay (LLNA) was accepted as an alternative stand-alone method. Here, we present data from German EU-notifications of new chemicals. Submission of significant numbers of LLNA reports started in 2003. Over time, the proportion of LLNA reports within totally submitted sensitization study reports in Germany increased to about nearly 50% in 2005. However, no further increase was observed thereafter. This may depend on still existing reluctance of notifiers, since the LLNA is presumed to produce an increased rate of false positives compared to guinea pig testing. Accordingly, some companies still did not establish the LLNA in-house and most of the studies are performed by contract labs. In one company, a non-radioactive LLNA was developed which considerably deviates from the standard LLNA, and makes it somewhat difficult to compare results. Regarding the technical performance and robustness of protocols, the LLNA is superior compared to GPMT, which demands more experience, resulting in more studies showing methodological flaws compared to the LLNA. In addition, LLNA results are often easier to evaluate compared to guinea pig data. In contrast to concerns that the LLNA would generally lead to on increase of positively evaluated substances, the proportion of substances classified as sensitizers remained in the same range. In conclusion, our experiences support the use of the LLNA for routine regulatory use. However, there exist substances with specific properties, which still may require testing in guinea pigs.

W 45 DISCORDANT RESULTS BETWEEN THE LLNA AND GUINEA PIG TESTS: SPECIFICITY *VS.* SELECTIVITY.

A. Mehling[2, 9], N. Ball[1, 9], H. Certa[8, 9], D. Eigler[6, 9], H. Esch[4, 9], C. Haux[3, 9], R. Kreiling[7, 9], K. Skirda[9] and G. Veenstra[5, 9]. [1]*Dow Europe GmbH, Horgen, Switzerland,* [2]*Cognis GmbH, D√É¬ºsseldorf, Germany,* [3]*Akzo Nobel Surface Chemistry AB, Stenungsund, Sweden,* [4]*BASF AG, Ludwigshafen, Germany,* [5]*Shell International BV, The Hague, Netherlands,* [6]*Goldschmidt GmbH, Essen, Germany,* [7]*Clariant Produkte (Deutschland) GmbH, Sulzbach, Germany,* [8]*SASOL Germany GmbH, Marl, Germany and* [9]*CESIO, Brussels, Belgium.* Sponsor: D. Basketter.

Although the local lymph node assay (LLNA), when using the [3H]-thymidine incorporation method described in OECD 429, gives reliable results for many substances, recent experience by Industry suggests it may overestimate or more rarely underestimate the sensitisation potential of some chemicals. Due to ethical aspects and animal welfare laws, comparative or repeated testing for regulatory purposes is normally not acceptable in the European Union. Therefore, defining unexpected results as false positives or negatives from an industry viewpoint can be difficult especially with regards to regulatory acceptance. From a consumer perspective, risk assessments could be based on inaccurate data. Published data and hitherto unpublished data from industry presented here, point to some limitations of the LLNA. Unexpected positive results were observed in LLNA tests performed with classes generally regarded to lack a substantial sensitization potential, e.g. surfactants, siloxanes and polyols. Most of these chemical classes were poorly represented in the original validation data set. Furthermore, discrepancies between other results obtained from guinea pig tests (GPTs), human experience and LLNAs were found. Various surfactants yielded unexpected positive results. High molecular weight polyols induced positive reactions although structurally similar substances did not sensitise in GPTs. Conflicting results were also obtained with some siloxanes, aliphatic hydrocarbons, fatty alcohols and unsaturated fatty acids. The presence of double-bonds in fatty acids as well as irritation may play a role but other mechanisms are feasible. These results indicate that the LLNA has limitations that require improved characterisation and understanding.

W 46 WHERE ARE WE NOW: TRUE AND FALSE POSITIVES AND NEGATIVES.

D. A. Basketter. *St John's Institute of Dermatology, St Thomas' Hospital, London, United Kingdom.*

The LLNA is now the de facto benchmark against which in vitro alternatives will largely be judged. Accordingly, it is important to be clear about the quality of this standard, for which a proper appreciation of true false positives and true false negatives is vital. This presentation will seek to provide a digest of the preceding material and draw out the main issues and conclusions. In particular, the local lymph node assay (LLNA) is being used increasingly in the identification of skin sensitising chemicals for regulatory purposes. In the context of new chemicals legislation (REACH) in Europe, it is the preferred assay. The rationale for this is that the LLNA quantitative and objective approach to skin sensitisation testing, allied with the important animal welfare benefits that the method offers. However, as with certain guinea pig sensitisation tests before it, this increasing use also brings experience with an increasingly wide range of industrial and other chemicals where the outcome of the assay does not always necessarily meet with the expectations of those conducting it. Sometimes, the result appears to be a false negative, but rather more commonly, the suggestion is that the chemical represents a false positive. We have here reviewed a number of instances where false positive and false negative results have been described and have sought to reconcile science with expectation. Based on these analyses, false positives and false negatives do occur in the LLNA, as they do with any other skin sensitisation assay (and indeed with all tests used for hazard identification), and that this occurs for a number of reasons. However, false positive results in the LLNA, as with the guinea pig maximisation test, can arise via failure to distinguish what is scientifically correct from that which is unpalatable, especially where classification and labeling is entirely hazard based. The consequences of this will be discussed, particularly in relation to the need to integrate both potency measurement and risk assessments into classification and labelling schemes which aim to manage potential risks to human health.

47 COMPUTATIONAL TOOLS AND TESTING METHODS: DELIBERATIVE AND JUDICIOUS USE IN RISK ASSESSMENT.

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Humans are exposed daily to chemicals in the environment, mostly as mixtures, through interaction with consumer products, through diet, and in occupational settings. The process of assessing the health risk associated with human exposure to such chemicals inevitably relies on a number of assumptions, estimations, and rationalizations. Out of necessity, computational methods are increasingly being used to meet the greatest challenges to extrapolate from conditions in experimental studies providing evidence of toxicity of the chemical to those anticipated in the environment. However, integrating these methods into useful decision-making tools involves a thorough understanding of the inherent assumptions, the associated databases, and a critical interpretation of the results because they tend to give a false sense of precision. This will be illustrated through the use of applications, at ATSDR, of structure activity relationships (SAR) to evaluate mutagenicity and developmental toxicity of Alkoxyethanols, and application of physiologically based pharmacokinetic (PBPK) modeling for derivation of minimal risk levels (MRLs) of methylene chloride and to define threshold for interactions of binary chemical mixtures.

Approaches and strategies that capture current biological knowledge, can help design model directed research, promote development of new in vitro methods, and integrate them with computational methods provide powerful alternatives and enable the scientific community to use them to address real life issues. These can be used to fill research needs without the expense of extensive laboratory or field testing. Parallel with the developments in the scientific world and our understanding of advantages and limitations of the computational methods "virtual toxicity testing" without animal use can be set as a long term goal of toxicology.

48 MODULATION OF GENE EXPRESSION BY BTEX: RISK ASSESSMENT IMPLICATIONS.

D. A. Sarigiannis, G. Cimino-Reale, E. Marafante, A. Collotta and A. Gotti. *Institute for Health and Consumer Protection, European Commission - Joint Research Centre, Ispra, Italy.* Sponsor: L. Birnbaum.

Benzene, toluene, ethylbenzene, and xylene (BTEX) are volatile monoaromatic hydrocarbons commonly found in gasoline and in outdoor and indoor air mixtures. Environmental monitoring studies demonstrate significant differences in the mean exposure of the general population. Although exposure to VOCs has been associated with health risks very little is known about the modulation of toxic responses by the exposure levels of BTEX and the variation in its composition. In this study we investigate the modulation of gene expression in vitro on human bronchoepitelial cells A549 and in vivo in the lungs of mice exposed i.tr. to two different BTEX mixtures representative of European cities. Human and Mouse Genome Survey Microarray and TaqMan Low Density Arrays by Applied Biosystems were used. Results from the in vitro studies demonstrated dose-response relationships for sub-toxic concentrations ranging from 10ng to 10 ug/l. The gene expression pattern correlates to different ratios of benzene/toluene in the BTEX mixtures. Similar results were obtained from in vivo studies on mice at 24 hours following i.tr. exposure. Increasing concentration of toluene was a major factor in modulating the expression of genes related to apoptosis, cell cycle control and proliferation, detoxification and inflammation. These results were confirmed by Quantitative PCR assays and are consistent with the output of a physiologically-based pharmacokinetic model of BTEX and its metabolites, which shows differences in both the transient and steady-state biologically effective doses of the BTEX components as a function of mixture composition.Our findings suggest that toluene plays a critical role in the toxicological response of the BTEX mixture. The composition of the mixture affects the bio-persistence of the primary compounds and their metabolites. This may

bear a strong impact on BTEX health risk assessment. For proper risk assessment we need to account not only for personal exposure levels, but also for differences in the chemical composition of the mixture.

49 EXPLORING THE BOUNDARIES OF ADDITIVITY: MIXTURES OF NADH-QUINONE OXIDOREDUCTASE INHIBITORS.

J. W. Boyd, J. B. Patrone, N. T. Boggs, H. T. Le and M. L. Theodore. *Eisenhower Research Center, Johns Hopkins University Applied Physics Lab., Laurel, MD.* Sponsor: M. Trush.

Mitochondrial NADH:quinone oxidoreductase (complex I) is composed of up to 46 subunits with a mass reaching 1000+ kDa, and is the largest and most complicated enzyme in the electron transport chain (ETC). Many complex I inhibitors target a single large binding domain with partially overalapping sites localized to the PSST subunit which couples electron transfer from the iron-sulfur cluster N2 to ubiquinone. In light of the shared binding site and the implied "common mode of action" of these inhibitors, our laboratory has tested the theory of dose-addition with HepG2 cells exposed to mixtures of three classic complex I inhibitors: rotenone, deguelin and pyridaben. We chose two isoeffective mixture rays, based upon the EC01 and EC50 values of the individual inhibitors, and examined all binary and tertiary combinations. Additionally, the impact of upstream potentiation (via glycolysis inhibition) on downstream additivity was tested with co-administration of 2-deoxyglucose in combination with mixtures of complex I inhibitors.

50 OPENREP: A COMMUNITY-BASED DELIBERATION PLATFORM FOR DEBATING DIOXIN AND DIOXIN-LIKE CHEMICAL TOXIC EQUIVALENCY DATA AND ASSIGNED FACTORS.

L. D. Burgoon and T. R. Zacharewski. *Toxicogenomic Informatics & Solutions, LLC, Lansing, MI.*

Toxic equivalency factors (TEFs) are used to assess the relative risk of mixtures of toxic polychlorinated dibenzo-p-dioxins, dibenzofurans and biphenyls relative to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The World Health Organization's International Programme on Chemical Safety (IPCS) has selectively used relative effect potency (REP) data reported in the literature to establish TEFs with limited input from affected stakeholders, advocacy groups and data providers. OpenREP was created to facilitate open and public deliberation, in order to capture community input using a wiki framework to support REP and TEF data presentation, stakeholder comments and facilitate the debate regarding the weighting of REP values, as well as the (re)assessment of current TEF values. In OpenREP each toxic congener has a Risk Assessment Dossier consisting of general chemical information, the current WHO TEFs, stakeholder-based recalculated TEFs, stakeholder discussion forums, and the in vivo and in vitro study summaries used to establish the current TEF values. General chemical information includes the chemical name, Entrez PubChem link, the IUPAC name, and structure information (canonical SMILES, and InChI structure codes). The in vivo and in vitro study summaries include REP values, assay system, route of administration, study quality weighting, and quality weighting justification sections. Links to the PubMed record for each citation are also listed. Stakeholders may also submit new literature for consideration. Curators will monitor the dossiers and discussion sections to ensure that posted data accurately reflect current stakeholder discussions regarding REP calculations and study weights. All stakeholders will be able to participate in the deliberation process, and the contents and deliberation materials will be freely available to government regulators and members of the WHO TEF committee. The underlying literature database will also be freely available to the scientific community.

51 A FRAMEWORK TO EVALUATE CHILD-ADULT DIFFERENCES IN INHALATION DOSIMETRY OF GASES: APPLICATION TO SELECTED SYSTEMICALLY-ACTING VOLATILE ORGANIC COMPOUNDS (VOCS).

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We have developed a framework and analytical approach for evaluating the relative tissue dosimetry in adults and children for systemic effects of inhaled gases. Mean internal dose in adults and children is compared, based on dose metrics for the appropriate chemical form. Such information can help to focus efforts for obtaining additional data and for more refined analyses for classes of chemicals where there is the greatest potential or likelihood of children being at greater risk. The results can be combined with information on variability, to evaluate the adequacy of default uncertainty factors for protecting children, or to determine the need for chemical-specific modifications to uncertainty factors. Case studies at steady state were conducted for systemic effects of VOCs to provide perspective on the potential range of internal dose in children at different ages (3 months, 1 year, 5 years, and 10 years) vs. adults for various combinations of physicochemical and metabolic characteristics. Calculations were conducted for the parent, reactive metabolite, and stable metabolite for blood:air partition coefficients of 1-50, hepatic extraction ratio of 0-1, and for high or low intrinsic clearance (1000 or 0.1 L/hr). Illustrative analyses were also developed on relative dosimetry for chemicals with flow-limited and enzyme-limited clearance, and those that show significant age-related variability in enzyme capacity. These analyses indicate that in most cases, the ratio of internal dose for children:adults for these chemicals is likely to be within a factor of 2.3 when the toxic moiety is the parent, and within a factor of 1.5 when it is a reactive metabolite. Ratios were generally ≤1 for stable metabolites, but higher ratios were obtained when the toxic moiety is a stable metabolite that is cleared efficiently in adults, but poorly in children. Overall, the framework provides a structure for evaluating age-related kinetic differences and to focus future research in this area.

52 AGE-RELATED DIFFERENCES IN SUSCEPTIBILITY TO CARCINOGENESIS III. TENTATIVE CLASSIFICATION FOR AGENTS WITH PUTATIVE NON-GENETIC MODES OF ACTION, AND USE FOR ANALYSIS OF AGE-RELATED SUSCEPTIBILITY DIFFERENCES.

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This paper first proposes a system of categories/groupings for nonmutagenic modes of action for carcinogenesis, and then tentatively applies this system for analysis of available age-related carcinogenesis bioassay data for six putative nonmutagenic carcinogens (amitrole, DDT, dieldrin, diphenylhydantoin, ethylene thiourea, and polybrominated biphenyls). The classification is of modes of action rather than individual carcinogens, because the same compound can affect carcinogenesis in more than one way. Basically, we categorize modes of action as: (1) co-initiation (facilitating the original mutagenic changes in stem and progenitor cells that start the cancer process) (e.g. induction of activating enzymes for other carcinogens), (2) promotion (enhancing the relative growth vs differentiation/death of initiated clones (e.g. inhibition of growth-suppressing cell-cell communication) (3) progression (enhancing the growth, malignancy, or spread of already developed tumors) (e.g. suppression of immune surveillance, hormonally mediated growth stimulation for tumors with appropriate receptors by estrogens) and (4) inherently multi-stage processes (e.g., "epigenetic" silencing of tumor suppressor genes). A priori, agents that act at relatively early stages in the process are expected to manifest greater relative susceptibility in early life whereas agents that act via later-stage modes will tend to show greater susceptibility for exposures later in life. The presentation will juxtapose these expectations with results of a quantitative analysis of data for the six study compounds.

53 TUMORS DERIVED FROM MANY DIFFERENT TISSUES MAY HAVE POLYCLONAL ORIGIN: EVIDENCE AND IMPLICATIONS.

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Few ideas have gained such strong acceptance as the concept that tumors are monoclonal in origin; i.e., start with a single mutated cell that goes on to accumulate additional mutations as a tumor develops. However, studies involving chimeras have directly demonstrated polyclonality in rodent and human colon tumors and Novelli and others identified a bias against the detection of polyclonality in X chromosome inactivation studies. Based on this information, the primary literature regarding X chromosome inactivation was reviewed. Given the bias against detecting heterotypy in X chromosome inactivation studies, studies were categorized as those that found no X chromosome heterotypy in any fraction of the tumors examined and those that found X chromosome heterotypy in at least some fraction of the tumors examined. Twenty-seven different tumor types were identified for which there is evidence of X chromosome heterotypy, while only four tumor types were identified for which there is no evidence of X chromosome heterotypy. Also reviewed were DNA-based approaches that quantify tumor-associated oncogene and tumor suppressor gene mutations within normal tissues and tumors. Again, such studies have generated data more consistent with polyclonal than monoclonal tumor origin. Specifically, initiated cells are more common in "normal" tissues than previously realized and, while tumors have higher levels of mutation than normal tissues, oncogene and tumor suppressor gene mutations frequently are present as sub-populations within tumors rather than as the pure mutant populations expected to develop from a single initiated cell. Thus, it appears a significant proportion of tumors may be initiated through short-range interaction between two or more mutant cells or clones of cells and it may be possible to model polyclonal tumor development based upon the frequency and spatial distribution of cooperating mutant clones.

54 MODE OF ACTION (MOA) UNCERTAINTY FOR "DUAL MODE" CARCINOGENS: IMPACT ON RISK ESTIMATED FROM NAPHTHALENE-INDUCED NASAL TUMORS IN RATS.

K. T. Bogen. *Exponent Health & Environmental, Oakland, CA.* Sponsor: G. Brorby.

Histopathology observations previously reported for rat nasal tumors induced by chronic, lifetime respiratory exposure to naphthalene indicate that if the mode of action (MOA) involved mutations by one or more genotoxic metabolites, such effects were very likely amplified by extensive cytotoxicity-induced nasal hyperplasia that occurred in nearly all rats exposed at 10 or 30 ppm concentrations (6 h/day, 5 day/wk). For such a dual MOA (DMOA) carcinogen, the USEPA Guidelines for Cancer Risk Assessment recommend judgment be used to compare and assess extrapolated risks using separate "linear" (genotoxic) vs. "nonlinear" (nongenotoxic) approaches, but allow the latter approach only if supported by a mechanistic model validated over a broad dose range. Absent such validation, the Guidelines effectively prevent MOA uncertainty from being considered, despite clear evidence of DMOA operation as in the case of naphthalene-induced nasal tumors. To illustrate how DMOA uncertainty can be addressed quantitatively in this case, an adjustment-factor approach was developed analogous to reference-dose procedures used for classic toxicity endpoints. Specifically, lower bounds on the magnitude of rat-tumor-type specific DMOA-related uncertainty were obtained using a mechanistic 2-stage cancer-risk model adapted to reflect the empirical link between genotoxic and cytotoxic effects of the most potent identified genotoxic naphthalene metabolites, 1,2- and 1,4-naphthoquinone. Tumor-specific adjustment factors were then used to reduce naphthalene cancer potency estimated by linear extrapolation (under the default genotoxic MOA assumption), to account for the DMOA that more likely pertains to this compound. By this approach, even when a "nonlinear" toxicokinetic model cannot be fully validated, the effect of DMOA uncertainty on low-dose risk can be addressed.

55 RESEARCH STRATEGY TO BETTER INFORM THE DECISION AS TO WHETHER A CARCINOGEN IS A "MUTAGENIC CARCINOGEN"

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The Environmental Protection Agency (EPA) has released for public comment a "Framework for Determining a Mutagenic Mode of Action (MOA) for Carcinogenicity". This, along with the 2005 EPA "Cancer Guidelines" and the "Supplemental Guidance for Assessing Early-Life Exposure to Carcinogens", solidify the importance of developing a research strategy to determine whether mutation is a key event in the etiology of specific tumors. As outlined, there are several levels of evidence that may be applied and/or required to conclude that a chemical either is, or is not, inducing tumors via a mutagenic MOA. The highest level requires demonstrating that the chemical induces mutation in one or more specific tumor oncogene(s) and/or tumor suppressor genes, a technically challenging burden of proof. The next level of evidence is a demonstration that the chemical induces mutations in surrogate genes in the tumor target tissue. Currently, in vivo transgenic mutation assays provide the best models for assessing mutation in tumor target tissues. Unfortunately, as generally conducted, in vivo transgenic mutation assays provide information for hazard identification, but fall short of fully informing cancer MOA. To assist with improving experimental design, we have conducted a review of the literature, and based on the behavior of chemicals generally thought to be mutagenic or nonmutagenic carcinogens, we propose a new experimental design that will optimally apply in vivo transgenic mutation models to the assessment of tumor MOA. Our design provides data to assess both temporality and dose response concordance, as outlined in the "Cancer Guidelines", and also provides an opportunity to obtain data for an array of other biological effects that may have relevance to nonmutagenic MOAs (or mixed MOAs) for the agent in question. The application of this research strategy will provide more definitive MOA information than is currently available from the weight-of-the evidence assessment of the routinely available genetic toxicology information.

56 FETAL TESTOSTERONE LEVELS ARE DIFFERENTIALLY AFFECTED IN MALE SPRAGUE DAWLEY AND WISTAR RATS AFTER *IN UTERO* EXPOSURE TO DIETHYLHEXYL PHTHALATE: A DOSE RESPONSE STUDY.

V. S. Wilson, K. L. Howdeshell, C. S. Lambright, J. Furr and L. Gray. *Reproductive Toxicology Division, U.S. EPA, ORD, NHEERL, Research Triangle Park, NC.*

Exposure to phthalate esters during sexual differentiation disrupts testosterone resulting in malformations of androgen-dependent tissues. We have found that gubernacular lesions are more prevalent in in utero diethylhexyl phthalate (DEHP)-treated Wistar male than in the SD rat offspring, whereas the SD rat displays a higher incidence of epididymal agenesis (Wilson et al, 2007. Tox Let). In addition, during gestation, fetal SD males had lower testosterone (T) hormone production and higher insl3 mRNA expression levels than Wistar males. The current study was designed to examine the effects of graded doses of DEHP on fetal androgen levels in these two rat strains. Rats were dosed with 0, 500, 625, 750 or 875 mg DEHP/kg/d on gestational days (GD)14-18 and fetal T production was measured on GD 18 (day of sperm = day 1). As hypothesized, untreated Wistar fetal males had higher T levels than SD rats and SD rats treated with DEHP at 500 and 625 mg produced 50% less T than did Wistar rats, indicating that the steroidogenic pathway is more affected in the SD strain than in the Wistar strain by phthalate treatment. Future studies will use lower doses of DEHP and examine fetal testis insl3 and steroidogenic gene expression in both strains. Taken together, these results are consistent with our initial observations, which suggested that the different malformation profiles produced by in utero phthalate treatment arise, at least in part, from strain differences in fetal Leydig cell function and the manner in which these cells respond to DEHP treatment. Disclaimer: This is an abstract of a proposed presentation and does not necessarily reflect USEPA policy.

57 INTERACTIVE EFFECTS OF DI-(2-ETHYLHEXYL) PHTHALATE AND DI-BUTYL PHTHALATE ON MALE RAT REPRODUCTIVE DEVELOPMENT: EFFECTS ON ANDROGENIC STATUS.

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Prenatal exposure to some phthalates results in several reproductive tract defects in male rats. We studied the effects of the coadministration of di-(2-ethylhexyl) phthalate (DEHP) and di-butyl phthalate (DBP) on intratesticular testosterone and androgen sensitive endpoints. Pregnant rats were treated by oral gavage from gestation day 13 to 21 with vehicle control, 150 mg DEHP/kg body weight (bw)/day (DEHP 150), 100 mg DBP/kg bw/day (DBP 100) or a combination of the two compounds (DEHP 150 + DBP 100 mg/kg bw/day). Based on previous dose-response studies, we predicted that the administration of these individual doses of DEHP and DBP would produce little, if any, changes on androgen sensitive endpoints. An additional group of rats was administered 500 mg DBP/kg bw/day (DBP 500) and served as a positive control for anti-androgenic effects. One set of dams (N=7-8/group) was sacrificed on pregnancy day 21. The anogenital distance corrected for body weight was recorded for male and female fetuses. The right testes of one or two male fetuses per litter were collected for analysis of intratesticular testosterone. Another set of dams (N=4-7/group) was allowed to deliver and the presence of nipples was examined in male pups on postnatal day 13. A significant reduction in testicular testosterone was observed in male fetuses exposed to DBP 500 or to the phthalate mixture. Anogenital distance was significantly reduced in male offspring of dams treated with DBP 100 or DBP 500, but animals exposed to the phthalate mixture ($p=0.08$) or DEHP alone ($p=0.95$) were unaffected. Nipple retention was only detected in animals exposed to DBP 500. Overall, coadministration of DEHP and DBP to pregnant rats significantly reduced fetal testicular testosterone at doses that did not produce any significant change in this endpoint when the compounds were administered alone. However, this alteration was not clearly associated with changes in external biomarkers of androgen development.

58 DI-BUTYL PHTHALATE TARGETS MULTIPLE SIGNALING MECHANISMS TO DOWN-REGULATE FETAL TESTOSTERONE BIOSYNTHESIS.

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The in utero hormonal environment is critical for proper development of the male reproductive system and there is growing concern that increases in various human male reproductive tract abnormalities, including falling sperm counts, hypospadias, cryptorchidism, and testis germ cell cancer are the result of exposure to environmental endocrine disrupting chemicals (EDC). In utero exposure of male rats to these EDCs targets fetal testicular testosterone biosynthesis leading to adverse effects on the developing male reproductive system. This lab has demonstrated that phthalate esters, a widely used class of EDCs, induce reproductive abnormalities in part by down-regulating the transcription of testosterone biosynthesis genes. However, the mechanism and upstream signaling pathways by which DBP inhibits transcription is unknown and explored in this study. This study identified altered protein-DNA interactions in down-regulated genes by quantitative in vivo DNase footprinting and chromatin immunoprecipitation. Using RNAi, gene expression of these factors was knocked-down in MA-10 and R2C Leydig cells. Cells were stimulated and steroidogenic gene expression was quantified with real-time RT-PCR to confirm the role of each factor in testosterone biosynthesis. Additionally, phosphorylation state of each factor was determined following stimulation and subsequent

phthalate exposure. Results indicate that the transcription factors sp1, SF-1, and c/ebp β are differentially bound following toxic and non-toxic phthalate exposure. Further, results suggest that these factors work coordinately through multiple or redundant mechanisms to regulate transcription, which reflects the importance of testosterone biosynthesis during fetal testicular development. Taken together, this study characterized the role of each transcription factor in the regulation of steroidogenesis and the signaling mechanism regulating its activity.

59 VINCLOZOLIN (V) INDUCES REPRODUCTIVE MALFORMATIONS AND INFERTILITY WHEN ADMINISTERED DURING SEXUAL BUT NOT GONADAL DIFFERENTIATION.

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Administration of the fungicide V during androgen-dependent sexual differentiation induces hypospadias, a vaginal pouch and undescended testes, among other effects in male rats. Recently, it was reported that ip administration of 100 mg V/kg/d during gonadal differentiation (GD8-15) induces epigenetic alterations that were transmitted over several generations (testicular histopathology, reduced sperm counts, infertility and tumors; Anway et al., 2005). However, two studies were unable to replicate the effects of V on the testis or fertility using either ip (Kawabe et al., 2007) or oral dosing (Schneider et al., 2006). Our study was designed to determine if oral 100 mg V/kg/d induced epigenetic effects in SD male rat offspring when administered during gonadal differentiation (GD8-15, n=10) or androgen-dependent sex differentiation (GD13-17, n=5). A vehicle control group (n=10) was dosed from GD8-17. AGD at birth was reduced in F1 males from 3.54 in controls to 2.68 mm by V from GD 13-17. Infant males treated with V retained female-like areolae/nipples (GD13-17 (11.0 areolae; 100% affected) and GD8-15 (2.7; 68%)). Only F1 males in GD13-17 group displayed hypospadias. When F1 males were mated at 165 days (n=24-25 pairs/group), fertility was reduced by V treatment on GD13-17 (48%) but not GD8-15 (92%) versus controls (80%). The effects of V on AGD and infant nipple retention were not transmitted to the F2 generation. F1 males are being necropsied and F2 males are being mated to produce an F3 generation. The histology of the testes will be evaluated and epididymal sperm counts will be measured in the F1, F2 and F3 generations to determine if vinclozolin treatment induced reproductive effects that were transmitted to subsequent generations. In summary, oral V treatment during gonadal differentiation did not affect the fertility of the F1 generation. In contrast, clear adverse reproductive effects were seen in the group exposed to V during androgen-dependent sexual differentiation.This abstract does not necessarily reflect USEPA policy

60 THE EFFECTS OF SUBACUTE ORAL EXPOSURE TO VINCLOZOLIN AND SEXUAL MATURITY ON 11β-HYDROXYSTEROID DEHYDROGENASE 2 ACTIVITY IN THE PORCINE TESTIS.

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Although the effects of endocrine disruption by vinclozolin (VCZ) and its metabolites on testicular development and function have been studied extensively in male rodents, little is known about the adverse effects of VCZ exposure on spermatogenesis in non-rodent mammalian species. Similarities between humans and swine in size and physiology make the boar a novel animal model for human risk assessment procedures involving the potential adverse effects of putative EDCs. Glucocorticoids are involved in the regulation of spermatogenesis by the initiation of apoptosis in the early stages of germ cell development, and intratesticular cortisol activity is modulated by the isoforms of 11β-hydroxysteroid dehydrogenase (11βHSD), especially 11βHSD2 in the boar testis. Since the observed adverse effects of VCZ exposure on spermatogenesis appear to be dependent on the stage of sexual maturation, it was hypothesized that subacute oral exposure to VCZ, as well as sexual maturity, would alter testicular 11βHSD2 activity in the boar. Eight, 8-week-old (prepubertal), nine, 7-month-old (peripubertal) and eight, 12- to 14-month-old (postpubertal) boars were each divided into two groups, which were orally exposed to either 0 or 100 mg VCZ/kg body weight for 14 days. Following euthanasia, sections of one testis from each boar were collected and flash-frozen in liquid nitrogen and stored at -80°C prior to analysis for 11βHSD2 activity using a validated radiometric assay. Statistical analyses were performed using ANOVA. Testicular 11βHSD2 activity was increased in VCZ-exposed pre-, peri- and postpubertal boars, as compared to similarly aged controls, but 11βHSD2 activity in the testis decreased as boars became more sexually mature. We conclude that subacute oral exposure to VCZ and stage of sexual maturity both modulate testicular 11βHSD2 activity in the boar; 11βHSD2 has a putative role in the regulation of cortisol activity and spermatogenesis within the porcine testis.

61 AKT1 PARTICIPATES IN THYROID-DEPENDENT GROWTH OF THE PERIPUBERTAL TESTIS.

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Transient neonatal hypothyroidism induced by exposure to the goitrogen, 6-N-propylthiouracil (PTU), prolongs Sertoli cell proliferation and differentiation, leading to a subsequent increase in adult testis size. Akt1 has been implicated in the regulation of proliferation and apoptosis in the testis, we sought to determine if Akt1 modulates germ and/or Sertoli cell proliferation and/or apoptosis following exposure to PTU. We exposed Akt1+/+, Akt1+/- and Akt1-/- mice to PTU (0.01% w/v) via the dams milk. At postnatal day 90, PTU-exposed Akt1+/+ and Akt1 +/- male mice exhibited a 23% and a 15% increase in testis weight. A trend toward increased testis size was observed in Akt1-/- males, but it was not significant. By PND90, daily sperm production was increased in PTU-exposed Akt1+/+ and Akt1-/- males by 20 and 16%, respectively. No increase in DSP was observed in Akt1-/- males. Consistent with the hypothesis that Akt activity is important to circumvent stress-induced apoptosis, postnatal day 25 TUNEL analyses revealed a significant increase in apoptosis in the testes of PTU-exposed Akt1+/- and Akt1-/- males. Western analyses showed that the Bax to Bcl-2 ratio was shifted toward a pro-apoptotic response in PTU-exposed Akt1-/- testes relative to controls. Transient neonatal hypothyroidism led to significant upregulation of Cyclin D1 and D3
mRNA levels which was not observed in Akt1 heterozygous and Akt1-deficient mice transiently exposed to PTU. In addition, wild type males exposed to PTU exhibited significant increases in the expression of genes associated with dividing Sertoli cells, thyroid hormone receptor alpha and Id2; whereas, PTU-exposed Akt1-/- mice exhibited reduced expression. In summary, the altered developmental pattern of expression of a number of major Sertoli cell genes reflects a prolonged mitogenesis and delayed maturation of Sertoli cells in neonatally hypothyroid animals and points to Akt1 as a critical early regulator of postnatal testis development affecting germ cell survival and Sertoli cell maturation.

62 PRENATAL AND LACTATIONAL EXPOSURE TO ETHINYLESTRADIOL, BUT NOT BISPHENOL A, ADVERSELY AFFECTS REPRODUCTIVE MORPHOLOGY AND SPERM PRODUCTION IN THE MALE LONG EVANS HOODED RAT.

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Many synthetic chemicals with endocrine-activity are released into the environment, including the oral contraceptive ethinylestradiol (EE) and the plastic monomer bisphenol A (BPA). EE and BPA are reported to impair reproductive organ development in laboratory animals; however, effects of lower doses of these chemicals have been debated. The goal of the current study was to determine whether relatively low oral doses of EE or BPA would alter the reproductive morphology and physiology of the male Long Evans (LE) hooded rat. Dams were gavaged with corn oil vehicle, EE (0.05, 0.15, 0.5, 1.5, 5, 15 and 50 μg/kg/day) or BPA (2, 20 and 200 μg/kg/day) during pregnancy through lactation from gestational day 7 to postnatal day (PND) 18. Male offspring were euthanized beginning at PND150, sera and organs were collected. Neonatal and adult body weight were significantly decreased in male offspring whose dams were exposed to 50 μg/kg/day EE. Developmental EE exposure reduced androgen-dependent tissue weights in male LE rats in a dose-dependent fashion; seminal vesicle and paired testes weights were reduced with doses of 5 μg/kg/day EE and greater. Epididymal sperm counts were also decreased by the 50 μg/kg/day EE treatment. Reproductive organ weight decreases occurred in the absence of a treatment effect on sera hormone levels, suggesting an organizational effect of EE on the male reproductive tract. Treatment with BPA did not significantly affect any male endpoint in the current study. In addition, EE doses of 5 to 50 μg/kg/day induced cleft phallus and decreased fertility in the female offspring of this study. Therefore, developmental exposure to oral micromolar doses of EE can permanently demasculinize the male reproductive tract and impair female fertility in LE rats. This abstract has been reviewed by the US EPA and does not necessarily reflect US EPA views or policy.

63 METABOLOMICS: APPLICATION TO THE STUDY OF PHTHALATES IN REPRODUCTION AND DEVELOPMENT.

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This study was conducted to evaluate the use of metabolomics for improving our ability to draw correlations between early life exposures and reproductive and/or developmental outcomes. Pregnant CD rats were exposed daily during gestation

(gd 14-21) to vehicle or to butyl benzyl phthalate (BBP) at a level (750 mg/kg) known to induce effects in the offspring and at a level (25 mg/kg) not expected to induce effects. Urine was collected for 24 h (on dry ice using all glass metabolism chambers) from dams on gd 18 (during exposure) and pnd 21 (3 wk after exposure), and from pnd 24 pups (~ 4 wk after dams exposure to vehicle or BBP). Traditional phenotypic anchors (e.g., anogenital distance, retained nipples, hormones) were measured in pups (between pnd 0 and pnd 26). Metabolomics of urine collected from dams (gd 18 and pnd 21) exposed to vehicle or BBP exhibited different patterns for endogenous metabolites. Even three weeks after gestational exposure, metabolic profiles of endogenous compounds in urine could differentiate dams that received the vehicle, low dose, or high dose of BBP. Metabolomic profiles of pnd 25 urine from offspring of dams exposed to vehicle, low dose or high dose of BBP were also significantly different. Metabolic profiles could differentiate male from female pups, pups born to dams receiving the vehicle, low or high BBP dose, and pups with measurable reproductive effects from pups with no observed effects. Metabolites significant to the separation of dose groups and the relationship with effects measured in the study were mapped to biochemical pathways for determining mechanistic relevance. The application of metabolomics to understand the mechanistic link between low levels of environmental exposure and disease/dysfunction holds a huge promise, because this technology is ideal for the analysis of biological fluids (e.g., urine, serum) in population studies, as well as the development of mechanistic insights from dose-response studies conducted in animal models. Supported by RTI International.

64 IMMUNOSUPPRESSION BY TBTO REVEALED BY GENE EXPRESSION PROFILING IN VIVO AND IN VITRO.

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The biocide and environmental pollutant bis(tri-*n*-butyltin)oxide (TBTO) causes thymus atrophy in rodents. Microarray analyses of the thymus of mice and rats and of rat primary thymocytes were performed in order to investigate if the immunosuppressive actions of TBTO could be detected by gene expression profiling and to elucidate the mechanisms of action. Transcriptional changes in murine thymus after exposure to a maximum tolerable dose of TBTO for 3, 7, or 14 days pointed primarily to reduced expression of cell surface determinants and T-cell receptor chains, suppression of cell proliferation, and interference with lipid metabolism. In a similar experiment in rats, only limited effects on gene expression of a lower dose of TBTO were found, even though thymus atrophy was observed. Transcriptional profiles in rat thymocytes directly exposed to TBTO (0, 0.1, 0.3, 0.5, and 1.0 µM) *in vitro* for 3 or 6 h showed regulation of lipid metabolism by TBTO at a low dose that did not yet result in disruption of cell functioning (cell viability and apoptosis). Higher doses caused regulation of apoptotic processes, which corresponded to present or subsequent thymocyte apoptosis observed phenotypically. The highest dose level tested additionally repressed mitochondrial functioning and immune cell activation. The difference in primary mechanism of action detected in our *in vivo* and *in vitro* studies is most likely a result of differences in experimental setup, and induction of apoptosis by TBTO may be an early effect that precedes inhibition of cell proliferation in time. Altogether, gene expression analysis was able to reveal known effects of TBTO and to extend the insight into its molecular mechanism of action at an early time point.

65 OVERLAPPING GENE EXPRESSION PROFILES OF IMMUNOTOXIC MODEL COMPOUNDS.

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In order to investigate gene expression changes that accompany immunotoxic effects of a set of model compounds in mice, a toxicogenomics approach was combined with assessment of macroscopical and histopathological changes in spleens and of modulation of immune function. Bis(tri-*n*-butyltin)oxide (TBTO), cyclosporin A (CsA), and benzo[a]pyrene (B[a]P) were administered to C57BL/6 mice at immunosuppressive dose levels for 3, 7, or 14 days. Acetaminophen (APAP) was also included in the study since indications of immunomodulating properties of this compound had appeared in the literature. Several compound-specific and overlapping effects were detected by microarray analysis of the spleen. TBTO for instance interrupted cellular respiration, and CsA and B[a]P inhibited immunological processes. All compounds induced xenobiotic metabolism. The most significantly affected process for all toxicants was cell division, which corresponded to the suppression of lymphocyte proliferation after application of a known contact sensitizer in an immune function assay that was adapted from the local lymph node assay. The immunotoxicogenomics approach applied in this study thus pointed to immunosuppression through cell cycle arrest as a common mechanism of action of immunotoxicants, including APAP. Genes related to cell division such as Ccna2, Brca1, Birc5, Incenp, and Cdkn1a (p21) were identified as candidates for indication of anti-proliferative effects of xenobiotics in immune cells in future screening assays. The results of the experiments also show the value of group wise pathway analysis for detection of more subtle transcriptional effects.

66 COMBINING TRANSCRIPTOMICS AND METABOLOMICS TO DELINEATE IMMUNOTOXICITY OF TRICHLOROETHYLENE.

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Chronic low-level exposure to the pollutant trichloroethylene (TCE) has been shown to promote autoimmune hepatitis and CD4+ T cell activation in MRL+/+ mice. The present study used transcriptomics and metabolomics to delineate liver events that skew disease development toward this organ in TCE-treated mice.

Female MRL+/+ mice were treated with 0.5 mg/ml TCE in drinking water for 26 weeks. These mice, unlike age-matched controls, developed early signs of autoimmune hepatitis including lymphocyte infiltration. This immune response was expected to correlate with expression of certain pro-inflammatory cytokine and chemokine genes. The "omics" evaluation of the liver showed that TCE upregulated the glutathione-mediated detoxification pathway. However, expression of most of the anticipated pro-inflammatory genes was not observed at the 26-week time point. Significant oxidative stress was also not detected at this time point. Instead, pathway analysis identified two new potentially immunoregulatory mechanisms. One of these involved signaling via the retinoid X receptor, a nuclear receptor that regulates liver inflammation. The second mechanism involved signaling via the IL-6R, a pathway with both pro- and anti-inflammatory consequences.

Taken together, it appears that if TCE-induced autoimmune hepatitis involves liver expression of traditional pro-inflammatory cytokines and/or chemokines, this must occur earlier rather than later in the exposure. Instead long-term exposure to TCE was associated with other pathways which may help maintain autoimmunity and/or attempt to attenuate disease development. In any case, combining transcriptomics and metabolomics in a pathway analysis provided novel information concerning the hepatotoxicity of TCE.

67 JUVENILE DEVELOPMENTAL IMMUNOTOXICITY (DIT)TESTING IN SD RATS: EFFECT OF DEXAMETHASONE ON INFLUENZA VIRAL CLEARANCE, NK ACTIVITY, CTL ACTIVITY AND T-DEPENDENT VIRAL-SPECIFIC IGM AND IGG ANTIBODY PRODUCTION (TDAR).

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Developmental immunotoxicity evaluations (prenatal, neonatal and/or juvenile) of pharmaceuticals and chemicals are critical to protect the pregnant mother and/or young child. The purpose of immunotoxicity testing is to obtain meaningful data for safety assessment and the ultimate predictor of immunotoxicity is host resistance assessment. The influenza viral-challenge model permits a multifunctional evaluation of the immune system and is one of the most thoroughly characterized host resistance models. In addition to viral clearance, functional immune measures can include: cytokines, macrophage activity, natural killer (NK) cell activity, cytotoxic T lymphocyte (CTL) activity, and influenza-specific IgM and IgG. These provide an evaluation of innate immunity by measuring macrophage or NK activity, an evaluation of cell-mediated immunity (CMI) by measuring CTL activity, and an evaluation of humoral-mediated immunity (HMI) by measuring influenza-specific IgM and IgG. The latter are T-dependent antibody response (TDAR) assessments since influenza is a T-dependent antigen. The purpose of this study was to evaluate the susceptibility of juvenile rats to the immunotoxicity of dexamethasone in the influenza host resistance protocol. In this study, weanling male and female Sprague Dawley (CD) rats were dosed daily by oral gavage with 0.06 – 2.0 mg/kg dexamethasone starting 7 days prior to infection with influenza virus and for 21 days post-

infection. Immunotoxicity was evaluated by measuring viral clearance on days 1, 2, 4, 8, and 21 post-infection. Mechanistic studies included measurement of NK activity on Day 2, CTL activity on Day 8, and T-dependent influenza-specific IgM and IgG on Days 8 and 21, respectively. Dexamethasone (0.06 mg/kg) treatment in the juvenile rats decreased viral clearance, suppressed NK and CTL activity (0.06 – 0.2 mg/kg), and decreased influenza-specific IgM and IgG (0.2 mg/kg).

68 DEVELOPMENTAL AND EARLY LIFE EXPOSURE TO TRICHLOROETHYLENE PROMOTES AN AUTISTIC PHENOTYPE IN MRL+/+ MICE: POTENTIAL ROLE OF OXIDATIVE STRESS IN NEUROIMMUNE DYSFUNCTION.

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Autism is a neurodevelopmental disorder estimated to impact 1 in 150 children in the US. This disorder is associated with oxidative stress and immune system alterations. In a recent study, one of the chemicals associated with autism was the environmental toxicant, trichloroethylene (TCE). We have developed a mouse model of environmentally-induced autism to examine the mechanism by which developmental TCE exposure promotes autistic-like behaviors. MRL+/+ mice were exposed to TCE in the drinking water. The offspring were evaluated at postnatal day (PD) 20 and 42 for immune function (thymus and periphery), measures of oxidative stress in the brain and thymus, as well as neurobehavioral effects. TCE-exposed male mice did not perform as well as controls in tests that measured sociability (i.e., nest building, resident-intruder). The behavioral alterations in the TCE-exposed mice were associated with a significant decrease in brain total glutathione, the major intracellular antioxidant. There was a significant increase in CD4+ T cell production of the proinflammatory cytokine, TNF-α, as evidenced by intracellular cytokine staining and flow cytometry. We propose that the observed oxidative stress in the thymus induces redox sensitive pathways that promotes pro-maturation in the thymus. The mature CD4+ T cells are further activated in the periphery to impact the brain and promote the autistic phenotype. These findings underscore the need for further study into potential environmental causes of human autism, and suggest that maternal exposure to chemicals like TCE may be an important factor for the development of this disorder.

69 BETA-NAPHTHOFLAVONE CAUSES AN AHR-DEPENDENT INHIBITION OF INVASION AND INTRACELLULAR MULTIPLICATION OF LISTERIA MONOCYTOGENES IN MURINE HEPATOCYTES.

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We recently reported a heretofore unknown role for the aryl hydrocarbon receptor in host resistance to listeriosis in mice. Hepatocytes are an important site for L. monocytogenes multiplication in vivo. In this study, we investigated whether activation of AhR in embryonic TIB73 and transformed Hepa-1 murine hepatocytes affects the ingestion and intracellular multiplication of L. monocytogenes. Western blot and real-time RT-PCR analyses indicated both cell types express AhR and are responsive to an AhR agonist, β-naphthoflavone (BNF). Treatment of TIB73 or Hepa-1 cells with BNF (10 μM) significantly inhibited the ingestion and intracellular growth of L. monocytogenes, which correlated with the up-regulation of CYP1A1. The inhibitory effects of BNF were both time and dose-dependent. Reduction of AhR expression in TIB73 cells by siRNA abolished the BNF-induced inhibition of L. monocytogenes uptake and intracellular growth. Inhibitory effects of BNF were also absent in AhR-deficient Hepa cells (CRL-2710), but were seen in ARNT-dysfunctional Hepa cells (CRL-2717). The prototypic AhR agonist-TCDD also dose-dependently inhibited the invasion and intracellular multiplication of L. monocytogenes in hepatocytes. Inhibition of ROS production with allopurinol (500 μM) or ascorbic acid (1 mM) did not alter the inhibitory effects of BNF. Neutralization of TNF-α or inhibition of NOS using L-NMMA (a general NOS inhibitor, 10 μM) or L-NIL (a selective iNOS inhibitor, 10 μM) also did not diminish the inhibitory effects of BNF. In addition, BNF (10 μM) neither directly killed nor altered the ratio of cell surface attachment to internalization of listerial cells. Taken together, BNF caused an AhR-dependent inhibition of ingestion and intracellular multiplication of L. monocytogenes in murine hepatocytes. These findings are consistent with our in vivo findings for the importance of AhR in optimal resistance to listeriosis in vivo.

70 A DEVELOPMENTAL IMMUNOTOXICITY STUDY IN THE JUVENILE RAT WITH CYCLOSPORIN.

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Cyclosporin has been shown to cause developmental immunotoxicity following administration to the juvenile rat (Barrow et al. 2006). A persistent depression of the primary antibody response was observed in adult rats that had received a relatively high dose of cyclosporin up to 4 weeks of age. The present experiment was performed with the objective of determining a no effect level for the adverse effects on the development of the immune system and to investigate whether such effects occur at dose levels that are otherwise non-toxic for the juvenile rat. The pups from 32 litters of SD rats were given 0, 1, 3 or 10 mg/kg/day of cyclosporin by gavage from 4 to 28 days of age. Each litter comprised one male and one female pup from each treatment group. Immune endpoints were determined in the pups at 4 and 10 weeks of age, including: lymphocyte subsets in spleen, liver and thymus; serum immunoglobulin titres; primary antibody response to sheep red blood cells (SRBC); organ weights; spleen cellularity and histopathology. The high dose level of 10 mg/kg/day resulted in a persistent impairment of the primary antibody response to SRBC at 10 weeks of age. Indications of systemic toxicity, including the death of 10/64 pups and severely depressed weight gain, were also observed at this dose level. The intermediate dose level of 3 mg/kg induced immune depression during the treatment period only, with no persistent effects in the adult. The low dose level of 1 mg/kg/day was a no effect level. It was concluded, therefore, that the adverse effects of cyclosporin on the development of the immune system in the rat were only manifest at a dose level that also induced systemic toxicity.

Reference: Barrow et al, Developmental Immunotoxicity Investigations in the SD Rat Following Pre- and Post-Natal Exposure to Cyclosporin. Birth Defects Research (Part B) 77: 430-437 (2006).

71 CHEMICAL TOXICITY DISTRIBUTIONS OF TRADITIONAL AND ALTERNATIVE ENDPOINTS: PARABEN EFFECTS TO AQUATIC ORGANISMS.

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Parabens are widely used antimicrobial agents found in thousands of pharmaceuticals and personal care products. Parabens are commonly employed because of their low toxicity in mammals. However, toxicity of parabens has not been established in aquatic organisms that could potentially be exposed to parabens released into aquatic systems. This is particularly interesting because previous studies identified select parabens as estrogen agonists. Standard and non-standard responses were evaluated in vivo with juvenile *Pimephales promelas* (fathead minnow) and *Daphnia magna*, and in vitro by hepatocyte cell cultures. Acute toxicity was determined for six different parabens (methyl, ethyl, propyl, isobutyl, butyl and benzylparaben) and their primary metabolite, p-hydroxybenzoic acid. LC50 concentrations ranged from 3.98 to 210.6 mg L-1 in *D. magna* and 3.27 to >125 mg L-1 in fathead minnow. Chronic reproduction in *D. magna* had lowest observable effects concentrations ranging from 1.5 to 6 mg L-1. Toxicity of these parabens was inversely related to their Kow value; subsequently, in vivo toxicity using standardized endpoints was attributed to narcosis. Utilizing toxicity benchmark concentrations, chemical toxicity distributions, a probabilistic hazard assessment technique, were developed to assess the probabilities of finding parabens or other compounds with similar mechanisms of action that elicit a response at or below a given concentration. These distributions demonstrated that at environmentally relevant concentrations there is limited acute hazard of parabens to crustaceans and fish.

72 SYNERGY OF MALATHION AND DIAZINON MIXTURES IN RAINBOW TROUT.

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Recent pesticide screens of urban streams feeding into Puget Sound quantified the anti-cholinesterases (anti-ChEs) carbaryl (C), diazinon (D) and malathion (M) at 0.2-0.4 ug/L. We studied in vivo mixtures of C, D and M, and in vitro inhibition of esterases in plasma and brain tissue using rainbow trout (Oncorhynchus mykiss). C, D and M exposures were 2.1, 4.3 and 2.2 ug/L, respectively, in US EPA static 96-hr toxicity tests. Reported 96-hr LC50s for C, D and M are 1,950, 90 and 200 ug/L, respectively. Individual chemical and binary and tertiary mixtures were assessed for toxicity, AChE and carboxylesterase (CaE) inhibition in brain, and CaE inhibition in plasma. There were no signs of toxicity or AChE inhibition in any single chemical exposure or in mixtures except the D/M and D/M/C groups where AChE was inhibited 85-88% and fish died and demonstrated abnormal swimming behaviors. Plasma CaE was inhibited 33-41% in C, M and C/M groups while D, D/C, D/M and D/M/C group CaEs were inhibited 91-98%. Brain CaE Inhibition

patterns were similar to plasma, with less inhibition in each group. Anti-ChEs generally act additively in mixtures. However, CaE inhibitors can synergize M toxicity due to the dependence on CaE hydrolysis of the M leaving group ester for M's generally low toxicity. In vitro inhibition studies (15 min, 25C incubation) with C, diazoxon (Do) and malaoxon (Mo) explained in vivo observations. Though brain AChE IC50s for Mo, Do and Ca (0.12, 4.8 and 5.3 uM) suggested high sensitivity to M exposure, CaE hydrolyzes M and decreases toxicity. Plasma CaE, the predominant trout CaE, was highly sensitive to Do inhibition, with Mo, Do and Ca IC50s of 0.087, 0.002 and 15.7 uM, respectively. These findings suggest that CaE inhibition at low D exposure levels synergizes the toxicity of M to trout by inhibiting M degradation by tissue CaEs, allowing increased formation of Mo and subsequent toxicity.

73 CHOLINESTERASE INHIBITOR PESTICIDE EFFECTS ON REPRODUCTION AND NEONATAL DEVELOPMENT IN NON-TARGET AVIAN SPECIES.

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Cholinesterase inhibiting compounds such as carbamates and organophosphate insecticides have been widely used in agriculture since the ban on organochlorines in the 1970s. Although these compounds are very effective against target insects and other pests, incidental morbidity and mortality on non-target species has also been well documented. Among the most vulnerable non-target species are birds. Despite efforts to minimize non-target exposure to these pesticides, birds continue to be exposed. This study was designed to investigate the impact of sub-lethal exposures to carbamate or organophosphate pesticides on avian species. In this study, homing pigeons (Columbia livia) were used as surrogate species to assess the differences in the effect of low-level and environmentally relevant doses of aldicarb (1.0 mg/kg), a carbamate, and chlorpyrifos (5.0 mg/kg), an organophosphate, two of the most widely used pesticides in California. The pigeons were utilized for trials on reproductive effects looking at fertility measurements, egg shell thickness, and post-hatch weight measurements. Pigeons were dosed by gavage with their respective treatment at least 7 days prior to being allowed to breed. While exposure to either compound had little effect on the number of eggs laid, exposure to aldicarb reduced the percentage of healthy offspring from 80% in the controls to 50% in the aldicarb treated group. Chicks were weighed 7 and 21 days after hatching. At 7 days, chicks born to pesticide exposed parents weighed 11% or 8% less than controls for aldicarb and chlorpyrifos birds respectively. By 21 days of age, there was no statistical difference in weights between the groups. Exposure to chlorpyrifos increased egg shell thickness, and exposure to aldicarb decreased it. These studies suggest that low-dose exposure to these chemicals can impact reproduction and neonatal development in these birds. These studies were funded in part by Nevada Agricultural Experiment Station Hatch Grant 0731.

74 MALE RAINBOW TROUT EXPOSED *IN VIVO* TO 17α–ETHYNYLESTRADIOL PRODUCE ANEUPLOID OFFSPRING.

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The environmental contaminant 17α–ethynylestradiol (EE2) is a potent estrogen found in many aquatic environments due to domestic sewage discharge. Although sexually mature male rainbow trout exposed in vivo to EE2 produce sperm consistent in concentration and motility with unexposed fish, reduced embryo survival at 19 days post fertilization (dpf) is observed. Previous results also indicate sperm from EE2 exposed males fertilize eggs normally. We therefore hypothesize that genetic damage is occurring during spermatogenesis causing reduced embryonic survival. Our objective was to determine whether male exposure to EE2 results in aneuploid embryos following in vitro fertilization. To reduce animal variation 10 clonal hybrid male rainbow trout were divided into two treatment groups with one exposed to 10 ng/L EE2 and one exposed to methanol only (solvent control) for 50 days using a flow-through exposure system. Following exposure semen was collected from sexually mature males in both treatment groups and cryopreserved in 0.5 ml cryostraws (10 cryostraws per individual). One hundred and fifty pooled outbred rainbow trout eggs were fertilized in vitro using two 0.5 ml cryostraw semen samples per male from three randomly selected males per treatment group. Cytological chromosomal analysis was performed on live embryos from each cross at 9 dpf with ten chromosomal spreads analyzed per individual. All spreads from embryos produced by fertilizing eggs with control male sperm exhibited a normal count of 62 chromosomes with embryos produced by fertilizing eggs from EE2 exposed male sperm having chromosome counts ranging from 59-64. Embryonic silver stain analysis was also performed revealing chromosome 20 trisomy in 27% of individuals. These data are consistent with the conclusion that the meiotic process in germ cells of male rainbow trout exposed to EE2 is compromised and results in significant numbers of aneuploid offspring that fail to survive.

75 CONGENER DISTRIBUTION OF POLYBROMINATED DIPHENYL ETHERS IN CHINOOK SALMON, LAKE TROUT, AND LAKE WHITEFISH FROM LAKE MICHIGAN.

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In the Great Lakes, it is estimated that the total concentration of polybrominated diphenyl ethers (PBDEs) in fishes is increasing exponentially every three to four years. The objectives of this study were to measure bioaccumulation of PBDEs in Great Lake fishes and biotransformation of BDE-209 in laboratory-dosed fish. Tissues (liver and muscle) of Chinook salmon, lake trout, and lake whitefish (n = 15 for each species and tissue type) were collected from Lake Michigan in April 2006 and juvenile lake whitefish were fed BDE-209 (control, 0.1, 1, and 2 μg/g/day) for 30 days. Lipid extraction was used to extract PBDEs from fish tissues. Gas chromatography/electron capture detector (GC/ECD) and gas chromatography/mass spectrometer (GC/MS) were used to quantify 11 PBDE congeners. There was a significant effect of fish species, tissue type, age, and maturity stage on PBDE concentrations. PBDE concentrations were highest in Chinook salmon, followed by lake trout and lake whitefish. Mature lake trout had higher concentrations of hepatic BDE-47, 99, 100, and 154; however, juvenile lake whitefish had higher BDE-153 in liver and BDE-209 in muscle. BDE-47 in liver and BDE-47, 99, 100, 153, and 154 in muscle decreased with body length only in Chinook salmon, whereas BDE-209 increased in muscle with increasing size. In relation to the BDE-209 feeding experiment, only nona- and deca-BDEs were detected and concentrations followed a dose-response relationship. Results from these studies suggest that all species are capable of debrominating BDE-209 into lower brominated congeners. This is of great toxicological significance, since lower brominated congeners are known to be more toxic.

76 IMPACT OF THIAMETHOXAM ON THE SERUM BIOCHEMICAL PARAMETERS IN CHANNA PUNCTATUS.

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The serum biochemical parameters of freshwater edible fish, Channa Punctatus, exposed to sublethal concentration of thiamethoxam were analyzed. An increase in glucose, lactate, bilirubin and creatine levels and a decrease in protein, non protein nitrogen (NPN), albumin and pyruvate levels were observed in the serum of C. punctatus exposed to 24, 48, 72 and 96 hours. Thiamethoxam significantly increased the specific activities of selected serum enzymes such as phosphatases, transaminases, dehydrogenases and creatine kinase. An increase in glucose and a decrease in protein content suggest enhanced glycogenolysis and proteolysis during thiamethoxam exposure. A decrease in pyruvate content with a simultaneous increase in lactate content reflects a metabolic shift from aerobic to anaerobic as a consequence of thiamethoxam stress. Similar decrease in serum albumin and increase in creatine and bilirubin content could be attributed to the renal failure and acute hepatic necrosis. An increase in the specific activities of serum phosphatases, transaminases and dehydrogenases suggests hepatic and renal dysfunction in the fish treated with thiamethoxam. An increase in the specific activity of creatine kinase could be attributed to the impairment of central nervous system during induced toxic stress. The maintenance of haemeostasis under stress conditions could be maintained by the development of some precursors.

77 PARENTAL AND TRANSGENERATIONAL EFFECTS OF EXPOSURE TO ENVIRONMENTAL STEROIDS IN TROUT.

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The contamination of aquatic environments with pharmaceutical and veterinary steroidal agents such as 17α-ethynylestradiol (EE2), trenbolone and melangestrol acetate (MGA) is a growing problem. Both EE2 and trenbelone are well established endocrine disruptors in fish, capable of causing reversal of sexual phenotype and

sterility among other adverse effects. We have studied the effects of EE2 and trenbolone exposure on HPG axis hormones and gene expression in sexually maturing trout and along with MGA, measured subsequent effects on fertility and overall embryonic developmental success. These studies used either out bred rainbow trout (O. mykiss) of mixed sex or male clonal hybrid trout exposed for 1 to 9 weeks to EE2, trenbelone or MGA. The timing of the exposures was varied according to the maturational state of the fish. The exposure concentrations ranged from environmentally relevant concentrations (0.8 – 10 ng/L) to 10X higher concentrations to establish dose-response and maximum intensity of the effect on embryonic development. In-vitro fertilization trials were performed with gametes from control and exposed fish. For male trout exposures, all trials used eggs pooled from 3-5 unexposed females and incubated with each semen sample at a sperm:egg ratio of 300,000:1. For female exposures, sperm from unexposed clonal hybrid male trout were used to fertilize eggs. All treatments were evaluated at 3-4 developmental timepoints including first cleavage, raised blastula, embryonic keel, and eyed stages, respectively. For EE2, the most sensitive exposure window appears to be when male trout are entering spermatogenesis where all treatments significantly decreased the number of eyed stage embryos relative to controls. Eggs collected from trenbolone exposed trout exhibited delayed developmental progress relative to controls. These results clearly demonstrate the trans-generational effects of select environmental steroids which can be the most sensitive reproductive endpoint in trout.

78 PHYSICO-CHEMICAL CHARACTERIZATION METHODS RELEVANT FOR TOXICOLOGICAL AND ECO-TOXICOLOGICAL EVALUATIONS OF NANOMATERIALS.

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The need for adequate and relevant particle characterization profiles for newly developed nanomaterials is becoming increasingly important in a variety of fields, including environmental, basic science, engineering, regulatory, and policy. Here, some of the critical material physico-chemical properties that are important when conducting toxicological assessments of nanoparticulates in eco-systems are described. In this regard, we have studied metal nano-colloids (silver), metal oxide nanoparticles (aluminum oxide, zinc oxide, silicon dioxide, and titanium dioxide), and carbon-based nanostructures (pristine and hydroxylated C60). By varying concentration (0.03 - 300 mg/L), changing solvent (medium) conditions in various aqueous media, and assessing nanoparticle characteristics at different time points (15 min, 1 – 96 hrs, 1 wk), we have studied changes in the aggregation potential of each nanoparticle system. Results showed that, depending on the nanoparticle system and its concentration, the aggregation potential for each of the nanoparticle-types changes over time and concentration. For instance, nano-sized silver particles aggregated to the same extent as fine-sized silver particles. At a concentration of 3 mg/mL (particle weight in cell culture media), the particles aggregated approximately 4 times to that of the primary particle size. As the concentration decreased, the extent to which nano and fine-sized silver particles aggregated also decreased. For the aluminum oxide (Al2O3) particle systems at the 3, 30 and 300 mg/mL concentration, the nano-sized Al2O3 particles aggregated to a larger size when compared to the fine-sized particles. The nano-sized Al2O3 has a primary particle size of 30 nm, but aggregated to 615 nm in media suspension. The fine-sized Al2O3 has a primary particle size of 190 nm and remained 190 nm in media suspension. Some systems show evidence of de-aggregation as well as changes in surface charge depending on solvent (medium) and time duration in suspension. This study presents our results regarding physico-chemical characterization of nano and fine-sized particles in aqueous media, and provides suggestions for relevant characterization defining the "nano" properties in reference to eco-toxicity assessments of particle systems.

79 OXIDATIVE DNA DAMAGE AND ITS REPAIR IN THE SPLEENS OF RATS EXPOSED TO ANILINE.

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Splenic toxicity of aniline is associated with iron-overload and oxidative stress. 8-Hydroxy-2'-deoxyguanosine (8-OHdG) is the most abundant oxidatively modified lesion in DNA, and 8-oxoguanine glycosylase 1 (OGG1) is one of the key repair enzymes that removes 8-OHdG and other oxidative guanine modifications. This study focused on examining DNA damage and repair activity in the spleen in an experimental condition that precedes a tumorigenic response. To achieve this, male SD rats were treated with aniline (0.5 mmol/kg/day through drinking water) for 30 days. Aniline treatment led to a significant increase in splenic DNA damage, evident from ~2.8 fold increase in 8-OHdG levels. DNA repair activity, measured as OGG1 base excision repair (BER) activity, increased ~1.3-fold in the nuclear extracts of spleens from aniline-treated rats. Real-time PCR analysis for OGG1 mRNA expression showed a 2-fold increase in the spleens of aniline-treated rats than the controls. Likewise, OGG1 protein expression in the nuclear extracts of spleens from aniline-treated rats was ~1.5 fold higher than the controls. Aniline treatment also led to stronger immunostaining for both OGG1 and 8-OHdG in the spleens, confined to the red pulp areas. It is evident from our studies that aniline-induced oxidative stress is associated with increased oxidative DNA damage. DNA repair pathways were also activated, but not enough to prevent accumulation of DNA oxidation products in genomic DNA, which could represent a critical step in the initiation of a mutagenic and/or carcinogenic response in the spleen. (Supported by NIH ES06476)

80 CYTOTOXICITY, OXIDATIVE STRESS, AND GENOTOXICITY IN HUMAN LIVER CARCINOMA (HEPG2) CELLS EXPOSED TO DINITROTOLUENES.

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Dinitrotoluenes (DNTs) [CH3C6H3(NO2)2] are nitroaromatic compounds that are produced industrially and released into the environment as a result of human activities. DNTs appear as pale yellow crystalline solids and exist as a mixture of 2 to 6 isomers, among which 2,4-DNT and 2,6-DNT are the most significant The primary targets of DNTs toxicity are the hematopoietic system, cardiovascular system, nervous system, and reproductive system. An association between DNTs exposure and increased risk of hepatocellular carcinomas and subcutaneous tumors in rats and renal tumors in mice has been established. This research was designed targeting the liver to assess the cellular and molecular responses of human liver carcinoma cells following exposure to 2,4-DNT and 2,6-DNT. Cytotoxicity was evaluated using the MTT assay. Upon 48 hrs of exposure, LC50 values of 245 + 14.72 μg/mL, and 300 + 5.92 μg/mL were recorded for 2,6-DNT, and 2,4-DNT, respectively. Lipid peroxidation is a mechanism of cellular injury in both plants and animals. Malondialdehyde (MDA) is an end product of lipid peroxidation. Results indicate that there is a strong dose response relationship between MDA production and DNT exposure. The comet assay, also called the 'Single Cell Gel Electrophoresis Assay', is a technique to detect DNA damage and repair at the level of single cells. Under a fluorescence microscope, cells with undamaged DNA appeared as intact comet heads without tails. Damaged DNA with single-strand breaks migrated in the direction of the anode, and this produced the appearance of a comet. The amount of DNA that migrates is proportional to the number of DNA single-strand breaks. Evaluation of the DNA "comet" tail shape and migration pattern allows for assessment of DNA damage. Using an image analysis system, relative amounts of DNA damage were obtained by measuring the length of the comet tail and the intensity of fluorescence in the tail. Results indicate that there is a strong dose response relationship between mean tail length, mean percent DNA damage, and DNT exposure.

81 3-METHYLINDOLE-INDUCED DNA DAMAGE IN PRIMARY NORMAL HUMAN BRONCHIAL EPITHELIAL CELLS.

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3-Methylindole (3MI) is a highly selective pneumotoxicant present in cigarette smoke. Human cytochrome P450 (CYP450) enzymes, such as 2F1 (CYP2F1), catalyze the dehydrogenation of 3MI to the reactive intermediate 3-methyleneindolenine (3MEIN). This metabolite has been shown to alkylate DNA and may induce both apoptosis and DNA damage. In addition, low concentrations of 3MI (ranging from 0.1 to 10 μM) have been shown to induce DNA damage in immortalized human bronchial epithelial (BEAS-2B) cells. However, the relative potency of 3MI to damage DNA in primary human lung cells in not known. The ability of low concentrations of 3MI to induce DNA damage in primary normal human bronchial epithelial (NHBE) cells was assessed using the alkaline comet assay. 3MI was shown to induce DNA damage at times (4 hours) and concentrations (0.1 to 10 μM) similar to that seen with treatment with the potent prototype DNA-damaging drug doxorubicin. 3MI-induced DNA damage was ameliorated following a 30-minute pre-treatment with the P450 suicide substrate inhibitor 1-aminobenzotriazole (1-ABT). Differential comet analysis at pH 8, 12.1, and 13.1 indicated that the majority of DNA damage 1 hour from 3MI exposure consisted of single strand breaks, contrasted to the double strand breaks observed upon exposure to the topoisomerase II inhibitor doxorubicin and the alkali labile site breaks observed upon exposure to the DNA alkylating agent N-nitroso-N-ethylurea (ENU). These results indicated that low concentrations of 3MI are bioactivated to intermediates that damage DNA in normal human lung cells by CYP450 enzymes. Thus, 3MI at concentrations less than 10μM is a potential human lung carcinogen. Supported by NIH grant # HL13645 from The National Heart, Lung and Blood Institute.

82 ASSESSMENT OF DNA DOUBLE-STRAND BREAKS AND g-H2AX INDUCED BY THE TOPOISOMERASE II POISONS ETOPOSIDE AND MITOXANTRONE IN V79 CELLS.

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Double-strand breaks (DSBs) are highly deleterious DNA lesions leading to chromosome aberrations and/or apoptosis. Topoisomerase II (TOPO II) poisons are antitumor drugs, that induce cancer cell death via this mechanism. TOPO II enzymes modulate DNA topology in regulatory processes such as chromosome condensation and DNA replication. Inhibition of TOPO II, particularly the α isoform (TOPO IIα), in dividing cells leads to the formation of stabilized cleavage complexes (SCC), generation of DSBs and ultimately apoptosis. The TOPO II poisons etoposide (ETO) and mitoxantrone (MTX) were studied in cultured V79 cells for 3 effects: 1) TOPO IIα stabilization using an immunoslotblot assay, 2) induction of DSBs using the neutral comet assay (NCA) and 3) phosphorylation of histone H2AX on Ser-139 (γH2AX), using an immunocytochemical and flow cytometric assays. Cells were treated with a wide range of drug concentrations up to toxic levels, as measured by cell counts, for 4 h in all three assays. In the case of ETO, a no observed adverse effect level and lowest observed adverse effect level for genotoxicity were determined; γH2AX levels and DSBs increased with ETO concentration but significant DNA damage was not detected until treatment with >0.5 μg/ml. DNA damage was dependent on the formation of TOPO IIα-SCC, which occurred at >0.1 μg/ml. In contrast, at low MTX concentrations (0.0001-0.001 μg/ml), induction of γH2AX was not accompanied by an increase in DSBs. Rather, NCA-detectable DSBs were only significantly increased when TOPO IIα-SCC were detected (>0.001 μg/ml). Thus, for both compounds, stabilization of TOPO IIα occurred at lower concentrations than induction of DSBs measured by the NCA. Our data comparing directly NCA and γH2AX induced by the TOPO IIα poisons ETO and MTX have shown that both assays are complementary in the assessment of DSBs generated as a result of TOPO IIα genotoxic effect.

83 DETECTION OF SINGLE STRAND BREAKS BY AN IMPROVED HYDROXYLAMINE COUPLED ALKALINE GEL ELECTROPHORESIS ASSAY.

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Detection of single strand breaks (SSBs) has been the focus of numerous genotoxicity screening assays. However, many of these approaches, including electrophoretic assays, are not well characterized and often generate unreliable data. For example, alkaline comet assays have the potential to give false positive results due to the simultaneous detection of SSBs and cleaved alkali-labile sites. While methoxyamine (MX) coupled alkaline gel electrophoresis (AGE) detects SSBs only, the neutralization of MX introduces high salt concentrations that may hinder complete DNA denaturation and obscure results. To improve SSB detection, we developed a semi-quantitative AGE assay coupled with *O*-(Tetrahydro-*2H*-pyran-2-yl)hydroxylamine (OTX). Like MX, OTX binds to aldehydic AP sites protecting them from alkaline incision, but in the absence of salts. As part of method optimization, genomic DNA was exposed to heat/acid conditions to produce AP sites. With increasing pH, AGE revealed that DNA denaturation was incomplete at pH≤12.1 and complete at pH12.8. To determine the optimal OTX concentration for protecting AP sites from alkaline cleavage, the DNA was exposed to a concentration gradient of OTX prior to denaturation with 100 mM NaOH (pH12.8) and AGE. Results showed that 30 mM OTX provided complete protection; thus, allowing the detection of only SSBs by AGE. As proof of concept, this approach was applied to DNA from polymerase beta proficient and deficient DT40 cells exposed to 1 mM MMS for 0.5-4 hours. As expected, SSBs were observed in a time dependent manner in both cell lines with more extensive damage present in the deficient cells. This OTX-coupled AGE SSB assay represents an accessible and facile approach for assessing SSB formation. With further development, this approach will allow for SSB quantitation and ultimately for its inclusion in a battery of assays to assess SSB formation and DNA repair capacity.

84 COUPLING CYTOTOXIC BIOMARKERS WITH GENOTOXICITY TESTS.

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In in vitro genotoxicity tests, the maximum concentrations of test compounds are determined by cytotoxicity, unless limited by solubility in solvents or culture medium. Cytotoxicity, which measures the degree of cell damage or death due to a chemical, varies in different test systems depending on the method to estimate it and the levels of toxicity that must be achieved. According to ICH S2A, in cytogenetic assays, the acceptable level of toxicity is defined as a 'significant reduction (>50%)' in cell number or culture confluency whereas mammalian cell mutation tests require relative total growth or cloning efficiency (CE) to be reduced by 80–90%. Conventional cytotoxicity assessment methods, including trypan blue exclusion assay and mitotic index, often underestimate cytotoxicity, revealing the need for developing reliable cytotoxic biomarkers to provide high sensitivity and certain indications of cytotoxic mechanisms in genotoxicity tests. Here, we introduced the ATP determination assay and Caspase-3 assay in combination with the Comet and micronucleus assays to test a number of cytotoxins, genotoxins and apoptosis inducers in in vitro mammalian cell culture systems. The ATP determination assay quantifies the ATP amount in metabolically active (live) cells, thus correlates well with cell number and viability. The Caspase-3 assay measures the activation of the Caspase-3 protease, which indicates the onset of apoptosis. The use of these cytotoxic biomarkers greatly aids in determination of maximum concentration of test articles in genotoxicity tests and provides a powerful tool to understand the mode of action (MOA) for the tested chemicals.

85 SODIUM DIMETHYLDITHIOCARBAMATE (DMDC) EFFECTS ON GNRH NEURONAL ACTIVATION AND THE LUTEINIZING HORMONE SURGE.

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In female rodents, noradrenergic input to the hypothalamus has a role in the secretion of gonadotropin-releasing hormone (GnRH) that triggers the surge of luteinizing hormone (LH). In these neurons, dopamine-β-hydroxylase (DβH) catalyzes the synthesis of norepinephrine (NE) and requires the presence of a copper cofactor. The thiram metabolite DMDC is a pesticide with metal chelating properties, and it along with related dithiocarbamates can reduce DβH activity and decrease NE. The effect is consistent with a suppression of the LH surge and normal ovulation. The present study examined a DMDC impact on the activation of hypothalamic GnRH neurons, indicated by the presence of the early gene product c-fos. A marked reduction or absence of c-fos positive neurons would be an essential link between effects on hypothalamic NE and the suppression of the LH surge. For this work, ovariectomized S-D rats received estradiol implants and 2d later injected SQ with progesterone (1200h), followed by a single IP injection (1300h) of 0.025, 0.05, 0.1 or 0.2 mM/kg DMDC. One group of animals was perfused at 1500h and the anterior hypothalamus sectioned and immunostained for GnRH & c-fos. The LH surge was assessed from tail nick blood sampled from a second group at 1400, 1600, 1800 & 2000h. Hypothalamic catecholamines were also determined in brains from a third group at 1500h. DMDC caused a dose-related decrease in the percentage of anterior hypothalamic c-fos positive GnRH neurons, with a partial reduction at 0.05 mM/kg (39.4% down to 23.5%) and a minimal presence of c-fos (<5%) at the two highest doses. These results correlated with a suppression of the LH peak, although the percentage decrease in c-fos neurons at 0.05 mM/kg did not reduce the area under the curve (AUC). The present data offer further evidence that the impact of DMDC on the LH surge is central in origin. Moreover, under these conditions, alterations in the surge peak, not AUC, more accurately parallel changes in neuronal activation. (This abstract does not reflect USEPA policy.)

86 DECREASED INSULIN SECRETION IN MURINE PANCREATIC BETA CELLS EXPOSED TO FLUORIDE.

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Fluoride ion is an important natural occurring contaminant, since it can be filtered towards deep water, due to its presence in minerals occurring at elevated concentrations in a number of parts of the world where it can be a significant cause of disease. Because fluoride is known to be an enzyme inhibitor interfering with calcium metabolism and enzyme mechanisms; the aim of this study was to evaluate if fluoride exposure at not cytotoxic concentrations was able to module the insulin secretion in mice pancreatic β-cells (βTC-6). Cells were exposed to NaF- at fluoride equivalent to 0, 0.007, 0.045, 0.180 and 2.26 mM by 12h; after this period cells were rinsed with PBS and after that, following tests were evaluated at stimulatory glucose concentration of 16.6 mM: i) flow cytometry analyses were realized to determine the mitochondrial membrane potential, using a membrane-potential-sensitive dye (JC-1); ii) superoxide anion generation was registered using dihydroethidium (DHE); iii) intracellular calcium concentration ($[Ca^{2+}]i$) was evaluated using a cell permeant dye fluo-3 AM; iv) kinase II dependent on Ca^{2+}-calmodulin (CaMKII) was evaluated by western blot. Results, showed a significant dose-response trend to decrease mitochondrial membrane potential and a increase superoxide generation in fluoride exposure; besides, $[Ca^{2+}]i$ it was significantly lower at 2.26mM, while CaMKII amount decreased at 0.180 and 2.26 mM. All these events produced a lower insulin

secretion in β-cells exposed to 2.26 mM of fluoride. In addition, there was observed a significant negative tendency on insulin secretion depending of fluoride concentration. In conclusion, β-cells exposed to fluoride decreased insulin secretion in response to glucose stimuli, this event was in relation with mitochondrial dysfunction which may caused the escape of electrons that readily leads to an increase in the generation of superoxide anion within the cell, and the inhibition of mitochondrial complex which in consequence decreased Ca^{2+} levels. This study was supported by Conacyt grant 50097.

87 DEVELOPMENT OF A RAT PRIMARY LEYDIG CELL ASSAY FOR DETECTION OF POTENTIAL ENDOCRINE SIDE EFFECTS.

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The ability to detect side effects of drug candidates is a critical part of a lead optimization paradigm. Endocrine side effects are challenging to monitor due to sex-specific sequelae in addition to complex feedback loops present in intact organisms. The focus of the present study was to develop an in vitro assay to monitor alterations in androgen production in a cell-based system. Briefly, a population of cells was isolated from rat testis using a collagenase-dispase digestion technique followed by centrifugation. The resultant population is enriched for Leydig cells. To assess responsiveness, Leydig cell cultures were treated with various concentrations of either forskolin or human chorionic gonadotropin (HCG) to stimulate production of testosterone (T), and prochloraz or ketoconazole to inhibit T production. Testosterone production was monitored by ELISA and parallel cultures were assessed to insure the test compounds were not cytotoxic. Finally a small panel of probes was developed to monitor alterations in the expression of mRNA for key steroid synthetic enzymes including CYP11a, CYP 17, and steroid acute regulatory protein (StAR). Treatment of Leydig cells with either forskolin or HCG resulted in concentration dependent increases in T production by 24 h. The maximal increase in T production was elicited by either 10 μM forskolin or 10 ng/ml HCG. Ketoconazole and prochloraz inhibited T production with approximate IC50s of 1 and 0.3 μM, respectively. TO901317 is an agonist of the liver X receptor (LXR) that is known to regulate expression of steroidogenic enzymes. Leydig cells were treated with 10 and 50 μM TO901317 and concentration dependent increases in mRNA for CYP11a, CYP17, and StAR were observed after 24 h. In summary, a primary Leydig cell assay was developed that is responsive to compounds known to both stimulate and inhibit production of testosterone. The Leydig cell assay is also able to detect changes in mRNA for key enzymes in the steroidogenic pathway.

88 THE EPIDERMAL GROWTH FACTOR RECEPTOR AS A POTENTIAL TARGET FOR OBESITY.

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The prevalence of overweight and obesity has increased sharply for both adults and children worldwide. WHO projects that by 2015, approximately 2.3 billion adults will be overweight, as defined as a body mass index (BMI) ≥ 25, and more than 700 million will be obese (BMI ≥ 30). This increase in fat mass is related to the food environment that is available. Factors contributing to this environment and the consequential toxic effects of overweight and obesity include large portions, visibility and accessibility of foods. Health risks attributable to this increase in fat mass include cancer, diabetes, heart disease and potential changes in susceptibility to environmental contaminants. Therefore, treatments are being pursued including inhibitors of fat uptake or stimulators of satiety signals. We have identified the epidermal growth factor receptor (EGFR), involved in normal adipocyte development, as a potential target for obesity treatment. Using a diet-induced obesity murine model, we found that genetic inhibition of EGFR using the $Egfr^{wa2}$ hypomorphic allele slows adipose mass deposition. Oral dosing of a small molecule inhibitor against the EGFR tyrosine kinase (AG1478) to diet-induced or polygenic obese mice recapitulated these results indicating that pharmacologic inhibition of EGFR may be used for obesity prevention. To determine how EGFR contributes to this effect, we deleted *Egfr* specifically in the central nervous system (CNS) of mice using the $Egfr^{tm1Dwt}$ conditional allele and a *GFAP-Cre* transgenic line. This demonstrated that EGFR activity is required in the CNS for fat deposition. MRI and DEXA analyses were used to measure the effects on fat mass and dissection of fat depots was used for validation of these *in vivo* measurements. Therefore, these results show that EGFR activity in the CNS is essential for the development of diet-induced obesity across diverse populations and that this effect can be recapitulated using small molecular inhibitors to EGFR kinase activity.

89 ENDOCRINE TOXICITY OF NITRATE/NITRITE.

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Nitrates and nitrites (jointly referred to as NO_x) are major global contaminants originating from combustion processes and the ubiquitous use of fertilizers in agriculture. Exposure to high levels of NO_x is associated with irritation of mucous membranes and methemoglobinemia. However, chronic exposure to low levels of NO_x has been hypothesized to cause toxicities associated with endocrine signaling disruption, perhaps through conversion to nitric oxide (NO), a potent intracellular signaling molecule. We tested this hypothesis using ecdysteroid signaling in the crustacean *Daphnia magna* as the model system. Our first objective was to establish whether nitrate, nitrite and the NO-generating chemical, sodium nitroprusside elicited toxicity to daphnids, indicative of altered ecdysteroid signaling. Exposure of maternal daphnids to each of these compounds resulted in concentration-dependent abnormalities in offspring consistent with an anti-ecdysteroidal mode of action. Next, we evaluated the ability of NO_x to undergo intracellular reduction to NO. We were unable to generate measurable amounts of NO via whole daphnids, homogenized tissues, or candidate oxido-reductases. However, NO was generated spontaneously from NO_x under conditions of transient acidification, as likely occurs during periods of embryo development. NO did not interfere with ecdysteroid signaling by lowering ecdysteroid levels in either maternal daphnids or their offspring. Presently, we are investigating NR1D3 (also called E75), a putative NO sensitive regulator of ecdysteroid signaling, as the target of NO toxicity. We propose that this putative downstream gas-sensor could be a potent target for NO perturbation of signaling. We have identified and cloned NR1D3 from *D. magna*. The NR1D3 recombinant protein is currently being expressed and evaluated for interactions with NO. Results from this study will advance our understanding of the potential threat posed by NO_x pollution to reproductive success.

90 IPRODIONE DELAYS MALE RAT PUBERTAL DEVELOPMENT, REDUCING SERUM TESTOSTERONE AND *EX VIVO* TESTOSTERONE PRODUCTION.

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Iprodione (IPRO) is a dichlorophenyl dicarboximide fungicide similar to the androgen receptor (AR) antagonist vinclozolin. The current studies were designed to determine if IPRO would delay male rat pubertal development like vinclozolin and to identify the mechanism(s) of action. We intend to use the dose response data obtained in the current study to determine how a mixture of IPRO and vinclozolin interact when administered during puberty. In the first study, Sprague-Dawley weanling rats were dosed by gavage with 0, 50, 100, or 200 mg/kg/day of IPRO from 23 to 51/52 days of age. The progression of preputial separation (PPS) was measured starting on day 37 and organ weights, serum hormones, and ex vivo testis hormone measured at necropsy (Blystone et al., in press). In the second study the hAR COS whole cell binding assay and an AR transcriptional activation with MDA-kb2 cells were used to determine if IPRO was an AR antagonist. In vivo, IPRO delayed PPS at 100 and 200 mg/kg/day and decreased androgen sensitive seminal vesicle and epididymides weights. As hypothesized, serum testosterone levels were decreased along with serum progesterone and 17α-hydroxyprogesterone whereas serum LH was unaffected. IPRO also reduced ex vivo testis production of testosterone, progesterone, and 17α-hydroxyprogesterone. In vitro IPRO bound to the AR and inhibited AR mediated transcription, but was considerably less potent than vinclozolin (binding IC50 = 86.0 μM vs 1.4 μM, respectively). Taken together, these results indicate that IPRO directly inhibits steroidogenesis in the rat testis, without affecting LH signaling. It is unclear at present however if IPRO's weak AR antagonism in vitro contributes to its anti-androgen effects in the pubertal male rat. *CRB supported through USEPA/NCSU Cooperative Training Agreement CT833235-01-0. This abstract does not necessarily reflect US EPA policy.*

91 MICROPLATE EDGE EFFECTS IN RANGE FINDING STUDIES USING THE LUMI-CELL® ER BIOASSAY: INCREASING ASSAY THROUGHPUT.

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Concern over the detrimental effects of endocrine disruptor chemicals (EDCs) has prompted the need for an effective high throughput system for detection of EDCs. Typically, the outer wells of a 96-well plate are not used for bioassay analysis due to edge effects, which have been found to reduce measured activity. This results in fewer available assay wells, decreasing analysis throughput by 38%. However, there

are very few published studies which actually document edging effects. We conducted 2 studies to examine the variability of the "outer" wells compared to that of the "inner" 60 wells. In the first study a dosing concentration of 2.5x10-5 μg/ml 17β-estradiol (E2) or 3.13 μg/ml Methoxychlor was plated in all 96 wells. An analysis of the outer ring and all successive inner rings were then compared to the inner 60 wells. None of the ring analysis or any of the outer rows and columns demonstrated any significant difference from the inner 60 wells when analyzed by grouped t-test. In the second study, a range finding assay was preformed on Bisphenol A (BPA) and Bisphenol B (BPB) (10 mg/ml each) using seven log dilutions. Each compound was then run vertically in the 96-well plate or horizontally within the normal inner 60 wells used in most assays. For both BPA and BPB, there was no significant difference in the response of the LUMI-CELL® ER bioassay using either placement. The data presented here using the ring and range-finder analysis, demonstrate the responsiveness and low variability of the outer wells of a 96-well plate in the LUMI-CELL® ER bioassay. After t-test analysis of all data there was no significant difference in any of the data analyzed. This data supports the use of the "outer" wells in screening or range finding studies, which will result in a 38% increase in sample throughput for the LUMI-CELL® ER bioassay. Supported by NIEHS SBIR grant ES10533-03 and Superfund Basic Research Grant ES04699.

92 EVALUATING DRUG-INDUCED THYROID EFFECTS AND SPECIES DIFFERENCES USING *IN-VITRO* THYROID CULTURE MODELS IN RAT AND HUMAN.

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We have established *in-vitro* models to investigate drug induced effects on thyroid function across species. In rat cultures, an entire thyroid lobe is used, and in human cultures, precision-cut thyroid slices are used. After pre-incubation of tissue in media alone, it is transferred to fresh media plus compound or vehicle for up to 72 hr. In order to evaluate perturbation of thyroid function, two anti-thyroid compounds were used to test the system, methimazole (MMI) and propylthiouracil (PTU). The effects of these drugs on the cultures are assayed by examining changes in the expression of genes involved in the thyroid hormone pathway. Specifically, these genes are thyroid peroxidase (TPO), deiodinase-1 (DIO1), sodium-iodide symporter (SLC5A), thyroglobulin (TG), cathepsin B (CTSB), megalin (LRP2), and TSH receptor (TSHR). For both compounds, concentration dependent changes in gene expression were statistically significant for several of these markers (TPO, DIO1, SLC5A, and LRP2). MMI and PTU significantly up-regulated TPO gene expression in rat thyroids at 24 hr at concentrations >10 μM. To correlate this response with enzyme function, the activity of TPO was measured and found to be inhibited at concentrations >1 μM for both compounds at 24 hr. Overall, the response to these compounds in human was weaker in comparison to rat. In conclusion, utilization of these novel thyroid models can facilitate in distinguishing compound effects on thyroid function and differences between species. This information will be valuable in understanding mechanisms of action and selecting compounds when effects on thyroid function are a safety concern.

93 TRICLOSAN ALTERS THYROID HORMONE HOMEOSTASIS *VIA* UP-REGULATION OF HEPATIC CATABOLISM.

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Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol) is a chlorinated phenolic antibacterial compound used in household and hygiene products. The structural similarity of triclosan to thyroid hormones, in vitro studies demonstrating activation of the human pregnane X receptor (PXR) and inhibition of sulfotransferases, and dose-dependent decreases in vivo of total thyroxine (T4) suggest adverse effects on thyroid hormone homeostasis. Current research tested the hypothesis that triclosan decreases circulating concentrations of T4 via activation of PXR, constitutive androstane receptors (CAR), and subsequent up-regulation of hepatic catabolism of hormones. Female Long-Evans rats (24 days old) were exposed po to triclosan (0-1000 mg/kg/day) for four days. Whole blood and liver were collected 24 hr after the last dose. Hepatic microsomal assays measured ethoxyresorufin-O-deethylase (EROD), pentoxyresorufin-O-deethylase (PROD), and uridine diphosphate glucuronyltransferase (UGT) enzyme activities. mRNA expression of UGT1A1, and sulfotransferase 1C1 were measured by quantitative RT-PCR. Triclosan caused a dose-dependent increase in PROD activity (up to 5-fold at 300 mg/kg/day). There was no biologically relevant change in EROD activity. T4-glucuronidation increased ~2-fold at 100 mg/kg/day. UGT1A1 mRNA expression increased in a dose-dependent manner (up to 2.2-fold at 300 mg/kg/day). These data suggest that triclosan-induced hypothyroxinemia is, at least partially, due to upregulation of hepatic catabolism. This abstract does not necessarily reflect the policy of the US EPA. K. Paul supported by NIEHS T32-ES07126.

94 EFFECT OF TRICLOSAN ON THYROID FUNCTION AND PUBERTAL DEVELOPMENT IN THE MALE WISTAR RAT.

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Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol) is a potent antibacterial and antifungal compound that is widely used in personal care products. A recent study found that triclosan exposure in the frog altered thryoid hormone homeostasis. The objectives of this study were to assess the effects of triclosan on thryoid function in a mammalian species and to also determine effects on pubertal development in the male rat. The rats were exposed to 0, 3, 30, 100, 200 or 300 mg/kg of triclosan by oral gavage from postnatal day (PND) 23 to 53. Preputial separation (PPS, a marker of pubertal development) was examined beginning on PND 33. Rats were necropsied on PND 53 and reproductive tissue weights were recorded. Serum was collected at necropsy for subsequent hormonal analysis. No effects of triclosan were observed on terminal body weight or the age of onset of PPS. However, mean serum testosterone (T) was significantly decreased at 200 and 300 mg/kg and androstenedione (A) was decreased at 100 – 300 mg/kg. Therefore, the NOEL for the effect on T was 100 mg/kg and the NOEL for A was 30 mg/kg. Triclosan also significantly decreased serum luteinizing hormone following 300 mg/kg, suggesting an effect at the pituitary level. No effects were observed in the reproductive tissue weights. In addition, triclosan significantly decreased serum thyroxin (T4) in a dose response manner at 30 mg/kg and higher (LOEL, 3 mg/kg was the NOEL). Triiodothyronine (T3) was also significantly decreased from 100 mg/kg to 300 mg/kg (NOEL 30 mg/kg). Mean liver weights were significantly increased at all doses which may indicate an induction of hepatic enzymes and subsequent clearance of T4. Pituitary weights were increased with all doses (LOEL-3 mg/kg). Further studies are needed to determine whether the suppression of LH is responsible for the decreased serum androgens. This abstract does not necessarily reflect U.S. EPA policy.

95 EFFECT OF A PERI-JUVENILLE EXPOSURE OF 2, 4-DICHLOROPHENOXYACETIC ACID (2, 4-D) ON THYROID FUNCTION AND REPRODUCTIVE DEVELOPMENT IN THE MALE WISTAR RAT.

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2,4-D is a widely-used chlorophenoxy post-emergence herbicide used to control broadleaf weeds. 2,4-D is registered for use on agriculture, fruit and vegetable crops and on turfs and lawns. It is currently undergoing re-registration considering the sensitivity of children and its endocrine disrupting potential. To better understand the reproductive/developmental and endocrine effects and to further evaluate the effects on the thyroid axis at low doses, we evaluated 2,4-D in the male pubertal protocol. 2,4-D was administered by oral gavage from postnatal day (PND) 23 to 53 at doses of 0, 3, 30, 100 or 200 mg/kg by oral gavage. Preputial separation (PPS, as a measure of pubertal onset) was assessed beginning on PND 33. Rats were necropsied two hours after the last dose on PND 53 by decapitation, serum collected for hormone analysis and reproductive tissue weights recorded. The highest dose resulted in a 14% decrease in terminal body weight and a significant delay in PPS (2.5d). All doses of 2,4-D (3 to 200 mg/kg) resulted in significant decreases in serum testosterone (T) and androstenedione. The androgen-dependent tissue weights were decreased at 200 mg/kg (seminal vesicle, ventral prostate and LABC muscle). Serum luteinizing hormone was significantly increased at 200 mg/kg, indicating an upregulation due to the decreased serum T. In addition, 2,4-D exposure resulted in a significant decrease in both thyroxine (T4) and triiodthyronine (T3), with a NOEL in both of 3 mg/kg. The delay in the onset of puberty and reduced weights of androgen-dependant tissues are likely due to the suppression of androgens. Therefore, further studies are in progress to determine the specific mechanism of action on the reduction of these steriods. This abstract does not necessarily reflect U.S. EPA policy.

96 EFFECTS OF ATRAZINE (ATR), DEISOPROPYLATRAZINE (DIA), AND DIAMINOCHLOROTRIAZINE (DACT) ON THE HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) AXIS IN FEMALE RATS.

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Previously we reported that a single dose of ATR herbicide stimulated HPA axis activation in the male rat while its primary metabolite, DACT, did so to a lesser extent. In this study, we evaluated the effects of ATR, DACT, and an intermediate

metabolite, DIA, on adrenocorticotropin (ACTH), corticosterone (C) and progesterone (P) in the female rat. Females displaying 4-day estrous cycles were predosed by oral gavage with vehicle (1% methyl cellulose, MC) for 5 days after which they received 4 consecutive doses of vehicle, ATR (12.5 or 75 mg/kg/day), or equimolar doses of DACT or DIA beginning on the day of estrus at 0900 (14/10 light cycle, lights on at 0500). Another group of regularly cycling females was dosed once with MC, 75 mg/kg ATR, or an equimolar dose of DACT or DIA at 0900 on proestrus. All rats were decapitated 15 minutes after the last dose. Plasma ACTH and serum C and P were measured by radioimmunoassay. Four doses of 75 mg/kg/day ATR significantly elevated circulating ACTH, C, and P compared to MC-treated controls. Multiple doses of 12.5 mg/kg/day ATR significantly elevated C, but not ACTH or P. Circulating ACTH, C, and P rose after multiple high, but not low, doses of DIA while neither exposure to DACT had an effect. A single high dose of ATR or DIA significantly elevated all hormones examined. Levels of ACTH and P were markedly greater than those measured after 4 doses while C levels were comparable between dose groups. One high dose of DACT had no effect on hormone levels. We conclude that ATR and DIA activate HPA axis activity in the female rat while DACT has no effect 15 minutes after dosing. Observed differences in hormone responses between single and multiple doses may reflect augmented adrenal sensitivity or higher baseline C after a 4-dose exposure. Additional studies will identify the primary target site of these effects, and will determine the role of adrenal steroids in mediating ATR-induced adverse effects on the reproductive system. This abstract does not necessarily reflect EPA policy.

97 EFFECT OF ACRYLAMIDE ON THE HYPOTHALAMIC-PITUITARY-THRYOID AXIS: GENOMIC, HORMONAL, AND HISTOLOGICAL INVESTIGATIONS IN MALE F344 RATS.

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Acrylamide (AA) is an important industrial chemical that is also formed during cooking of many starchy foods. Lifetime exposure of male and female F344 rats to AA produces tumors in several organs including thyroid, mammary, testicular mesothelium, and the central nervous system. The tumor sites observed in rats have prompted the hypothesis that AA induces cancer by endocrine perturbations through dysregulation of dopamine, thyroid hormones, and gonadotrophins. The present study examined in a systematic way the effects of AA in thyroid, pituitary, and hypothalamus (HPT axis) by measuring neurotransmitters, serum hormones, target organ histology, and gene mRNA levels. Male F344 rats were exposed to AA (approximately 2.5, 10, 50 mg/kg bw/d in drinking water) for 14 d. No changes were observed in hypothalamic or pituitary neurotransmitters, including dopamine, serotonin, and their metabolites. A small but significant diminution of serum T4 was observed at the high dose but no significant changes in TSH were observed. No dose of AA affected serum levels of TSH or T4 following a TRH challenge. No changes in thyroid or pituitary histology (H&E) were observed nor was there evidence for increased cell proliferation measured by Ki67 or PCNA immunoreactivity; actually, dose-related decreases in cell proliferation were observed. No significant changes were observed in the expression of genes involved in hormonal function of the hypothalamus (TRH, somatostatin, vasopressin, growth hormone releasing hormone, melanin-concentrating hormone), the pituitary (TSH, pre-growth hormone, LH, POMC, pre-prolactin), and the thyroid (TSH, thyroglobulin, thyroid peroxidase, sodium-iodide symporter) using cDNA macroarrays covering 2400 genes. These observations suggest that disruption of the HPT axis by AA is not involved in the neoplastic transformation of these endocrine tissues.

98 THYROID HORMONE STATUS IN ADULT RATS AFTER AN ORAL DOSE OF PERFLUOROBUTYRATE (PFBA).

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Perfluorobutyrate (PFBA) is a perfluorinated carboxylate formed by industrial synthesis and the metabolism and hydrolysis of certain chemicals. PFBA has been detected in surface waters and in public and private wells at up to low ng/mL concentrations. A 28-day oral toxicity study with PFBA in rats has observed: (1) decreased total and free thyroxine (TT4 and FT4) in serum at the end of treatment without a compensatory rise in thyrotropin, or TSH; and (2) hypertrophy and hyperplasia in liver and thyroid follicular epithelium. All the effects were seen in male rats only and were reversed after 21 days of recovery.
Because the clinical diagnosis of primary hypothyroidism is based on elevation of TSH, the reported reductions in serum thyroid hormone without a compensatory increase in TSH do not fit the profile of a hypothyroid state.
We hypothesized that PFBA can compete and displace FT4 from serum binding proteins. The reduction of serum TT4 and FT4 could be due to the higher turnover of displaced FT4. To test the hypothesis, the effects of PFBA on TH status were investigated over a 24-hour time period following a single oral dose of PFBA. Adult male SD rats (10 – 15 /group) were given 30 mg/kg PFBA ammonium salt (the mid-dose in the 28-day study) or vehicle (DI water) and sacrificed at 2, 6, 24, and 72 hours post-dose. Sera and liver were harvested and flash frozen immediately. Serum samples were analyzed for TT4 (chemiluminometric assay), FT4 (equilibrium dialysis followed by LC/MS-MS), and TSH (rat specific RIA).
Compared to controls, TT4 was significantly reduced over the 72 hours duration with PFBA. While FT4 were similar to control at 6 and 72 hours, it was significantly increased at 2 hours (by 29%) but decreased at 24 hours (by 31%). Mean TSH activities were not statistically different from controls at 24 and 72 hours but were reduced at 2 and 6 hours with statistical significance. These results demonstrate that thyroid status was not suppressed following PFBA administration.

99 THE EFFECT OF SULFATHIAZOLE ON THYROID-PITUITARY HORMONAL SYSTEM IN PREPUBERTAL RATS AND IN RAT PITUITARY AND HELA-TRE CELLS.

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Sulfathiazole is effective against a wide range of pathogenic microorganisms. It is used for the prevention or treatment of bacterial infectious diseases and the improvement of feed efficiency of livestock animal. Sulfathiazole has been reported to induce dysfunction of thyroid-hypothalamus-pituitary axis and its acceptable daily intake (ADI) and maximum residue limit (MRL) could not allocated because of the lack of data on the hormonal effects of sulfathiazole. This study was performed to investigate the potential toxic effects of sulfathiazole focusing on thyroid-pituitary hormonal system in prepubertal male rats and in rat pituitary and HeLa-TRE cells. In in vivo study, 28 days old Wistar male rats were orally administrated with sulfathiazole 0, 5 and 20 mg/kg bw for 2 weeks. Body weights were not affected by all doses of sulfathiazole. Organ weight of thyroid glands was increased but that of spleen decreased. All hematological indices were not significantly different from control, but the contents of total cholesterol, LDL-cholesterol, LDH and growth hormone (GH) were significantly increased but TG was decreased at the highest dose of sulfathiazole. Free T3 and T4, Total T4 and TSH contents in serum were significantly reduced. Rat pituitary cell (CCL-82.1) and Hela-TRE cells were exposed to sulfathiazole for 48hrs and for 24 hrs, respectively, to know the activity of sulfathiazole to thyroid hormone receptors (TR) and thyroid hormone responsive element (TRE). In rat pituitary cells, sulfathiazole decreased the release of GH into medium and inhibited the synthesis of GH induced by T3 (250pM). In HeLa cells expressing human TRE, sulfathiazole did not show binding activity to TRE except at the highest dose (100uM) but inhibited completely the binding of T3 (100pM) to TR or TRE. These results indicate that sulfathiazole induce thyroid and pituitary hormonal disruption in vivo, which are explained that sulfathiazole impact directly on thyroid receptor or TRE as complete antagonist against thyroid hormone.

100 IN VITRO METABOLISM OF THYROID HORMONES BY RECOMBINANT HUMAN UDP-GLUCORONOSYLTRANSFERASES AND SULFOTRANSFERASES.

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Endocrine disruptors can decrease thyroid hormone levels via the induction of hepatic uridinediphosphate-glucoronosyltransferases (UGTs) and sulfotransferases (SULTs). Due to their ability to catalyze glucuronidation and sulfation of hormones and xenobiotics, UGTs and SULTs play an important role in hormone catabolism and xenobiotic detoxification. However, it is unclear if induction of hepatic conjugation alone is responsible for the decrease in circulating thyroid hormones by xenobiotics. This study compares the catalytic glucuronidation and sulfation of thyroxine (T4) and triiodothyronine (T3) using recombinant human UGTs and SULTs. All UGTs examined catalyzed T4 glucuronidation, but UGT1A8, only found in the gastrointestinal tract, had an activity nearly 4-fold higher than the other UGT isoenzymes. T3 glucuronidation was also catalyzed by all UGTs examined, but activity levels were much lower than UGT-T4 activity. UGT1A8 and UGT2B4 catalyzed T3 glucuronidation with activity levels as much as 8-times higher than some of the other UGT isoenzymes. All SULTs examined catalyzed T4 and T3 sulfation, but overall T3 sulfation activity was greater than T4 sulfation activity. T4 sulfation activity of SULT1E was as much as 180-times higher than some of the other SULT isoenzymes examined whereas T3 sulfation activity of SULT1E was as much as 26-times higher than other SULTs. These data suggest that certain UGT and SULT isoenzymes may be more involved in thyroid hormone metabolism than others. In addition, extrahepatic isoenzymes such as UGT1A8 may play key roles in decreasing circulating thyroid hormones (This is an abstract of a proposed presentation and does not necessarily reflect USEPA policy.)

101 EFFECTS ON HEPATIC DEIODINASE 1 AND THYROID HORMONE LEVELS IN PERINATALLY EXPOSED RATS TO A PBDE COMMERCIAL MIXTURE.

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A commercial PBDE mixture, DE-71, mostly tetra- and penta-bromodiphenyl ethers had been commonly used as a flame retardant in polyurethane foam. These congeners are ubiquitous in the environment and human tissue. Previous developmental studies have shown exposure to PBDEs may affect thyroid hormone levels by inducing hepatic uridinediphosphate-glucoronosyltransferase (UDPGT) and transthyretin (Ttr) binding. This study examines effects of DE-71 on enzymes and genes related to thyroid hormone disruption at different developmental time points in male rats. Pregnant Long-Evans rats were gavaged at 0, 1.7 (low), 10.2 (mid) or 30.6 (high) mg/kg/day of DE-71 in corn oil from gestational day 6 to postnatal day (PND) 21. For pups at PND 4, 22 and 60 serum was collected for thyroid hormone levels and livers were analyzed for enzyme activity and mRNA expression (RT-PCR). Serum total T4 concentrations were unchanged at PND4 but decreased 50% (mid) and 80% (high) at PND22. UDPGT activity increased 95% (high) while T4 levels decreased 50% (mid) at PND4 and 22. This indicates a separate mechanism may contribute to serum T4 decreases. At PND4, hepatic deiodinase 1 enzyme activity was significantly decreased 60% at all doses, while on PND22 it decreased 55% (high) and returned to basal levels at PND60. At PND4, mRNA was reduced 40% (mid) and 50% (high) while at PND22 it decreased 30% (high) with no significant differences seen at PND60. This study demonstrates perinatal exposure to DE-71 alters activity and gene expression of deiodinase 1 at the lowest dose even though T4 levels were not decreased until PND22. Thus, the alterations in enzyme activity and gene expression are dose-and time-dependent but may not necessarily correlate with decreases in circulating thyroid hormone. (This abstract does not reflect USEPA policy. Supported in part by NIH T32 ES007126)

102 A 10-DAY STUDY COMPARING THE EFFICACY AND TOXICITY OF GLYCOPEGYLATED™ AND NON-GLYCOPEGYLATE HGH FOLLOWING SUBCUTANEOUS ADMINISTRATION IN HYPOPHYSECTOMIZED FEMALE RATS.

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GlycoPEGylation of peptides is an effective method to improve the PK profile of protein therapeutics. The purpose of the current study was to determine the efficacy and toxicity following subcutaneous administration of GlycoPEGylated and non-GlycoPEGylated hGH compounds in hypophysectomized female rats. A total of 65 rats were evenly divided into 13 treatment groups. Group 1 rats were injected with the vehicle once on study day (SD) 1. Group 2 rats were injected with non-GlycoPEGylated hGH once on SD 1 at a dose of 180µg/rat. Group 3 rats were injected with the same non-GlycoPEGylated hGH at 30µg/day for 10 days. Rats in Groups 4-13 were injected with different formulations of GlycoPEGylated hGH once on SD 1 at 180µg/rat. Blood samples for serum harvesting were collected at various timepoints (SDs -3,1,2,4,7,10) for IGF-1 analysis. Body weights of the rats were measured daily. In addition, daily postdose clinical observations were conducted on all rats to detect potential adverse signs or symptoms. All rats were euthanized on SD 11. The results of the study showed that in general, hypophysectomized rats treated with a single subcutaneous injection of GlycoPEGylated hGH (180µg) resulted in 1) enhanced secretion and prolonged serum level of Insulin Growth Factor-1 (IGF-1) and 2) greater positive body weight gains compared to rats administered either a single 180µg injection or ten 30µg daily injections of non-GlycoPEGylated hGH. In addition, none of the rats showed adverse reactions following administration of any formulations of the hGH. In conclusion, a single injection of the different formulations of GlycoPEGylated hGH was more effective than non-GlycoPEGylated hGH, administered either as a single or repeated injections, in stimulating IGF-1 secretion with a corresponding increase in body weight in hypophysectomized rats.

103 EFFECTS OF 4-*TERT*-OCTYLPHENOL ON *XENOPUS TROPICALIS* IN A LONG TERM EXPOSURE.

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Amphibian species are useful indicators for the effects of endocrine disruptor exposure in the environment. An experiment was conducted to determine the effects of the estrogenic endocrine disruptor, 4-*tert*-octylphenol (OP), on growth and reproductive parameters in *Xenopus tropicalis* in a flow through diluter system. Frogs were exposed to 1 µg/L, 3.3 µg/L, 11 µg/L, and 36 µg/L OP, at 34 frogs per tank, 3 tanks per concentration, and 9 tanks of unexposed frogs were used as controls. The frogs were exposed starting from stage 46 of metamorphosis through adulthood. At stage 65, approximately 6 to 7 weeks of exposure, 10 froglets per tank were randomly selected for blood collection for serum vitellogenin (VTG), sex determination by gross morphology, and gonadal histology. An induction of VTG in both males and females was seen in the 36 µg/L OP exposure compared to the control. Since VTG is an estrogen inducible yolk protein expressed at high levels in mature females, and stage 65 froglets should not have measurable VTG levels, this indicates that 36 µg/L OP was sufficient to stimulate early production of VTG. A significant increase in body length and weight for the 1 µg/L OP exposure compared to control was observed, but there was no difference in sex ratio for any of the OP concentrations. At the final necropsy, serum VTG, estradiol, testosterone, and T4 were measured, gonad, liver, kidney and thyroid tissues for histological examination were collected, and sperm counts, oocyte number, and stage were determined.
Opinions, interpretations, conclusions, and recommendations are those of the author and not necessarily endorsed by the U.S. Army.

104 EFFECT OF ARSENIC ON ESTROGEN STIMULATED PROLIFERATION IN HUMAN BREAST TUMOR-DERIVED T47D CELLS.

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Arsenic (As) contamination has generated considerable public health concerns both in the US and world wide. Gender differences have been reported regarding arsenic methylation and in arsenic-related skin lesions in humans and in tumor incidence in mice. As has been shown to bind to human estrogen receptor-alpha (ERα) at the hormone binding region and to influence estrogen-related gene expression, suggesting an estrogenic mode of action. Long term estrogen exposure is a major risk factor associated with "spontaneous" breast cancer. We hypothesize that exposure to environmental arsenic may modify the effect of endogenous estrogen on breast cancer cell proliferation. Using the ER-positive breast cancer derived T47D cell line, we first examined the dose-response relationship of 17-β-estradiol (E2)-stimulated cell proliferation as detected by BrdU-incorporation and sulforhodamine B assays. Two E2 concentrations, 10 pM (EC20) and 100 pM (EC80), both within the range of physiological levels, were used to study the effects of As and E2 each alone and in mixture. Cells were treated for 48 hrs with As (sodium arsenite) at 0, 0.1, 1, and 10 µM, either alone or in mixture with EC20 and EC80 concentrations of E2; As was added 30 min prior to E2. As itself possessed a weak stimulatory effect on cell proliferation and only at concentrations greater than 1 µM. In contrast, in mixture with 10 pM (EC20) of E2, As had a unique bi-phasic effect, which was synergistic at 0.1 µM but inhibitory at 1µM or higher. However, in mixture with 100 pM (EC80) of E2, As at all concentrations only had inhibitory effects. Cell proliferation stimulated by As alone and the As-E2 mixture was inhibited by the antiestrogen 4-hydro-Tamoxifen. To understand the molecular mechanisms of these complex effects, As-activated ER genomic and non-genomic signaling, as represented by estrogen-responsive gene expression and mitogen-activated protein kinase phosphorylation, are currently under investigation. Supported by Grants CA77550 and P30 ES03819.

105 MEASUREMENT OF MULTIPLE TYPES OF ENDOCRINE ACTIVITIES IN WATER.

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The presence of estrogen hormones and estrogen-mimicking chemicals in the environment has been subject of a large research effort worldwide for at least a decade. The presence of these compounds in surface water, from which also drinking water is prepared, gave concerns on the quality of this drinking water. We have therefore performed several studies on endocrine activity in drinking water and its sources. Estrogenic activity was measured with the ER-CALUX in the rivers Rhine and Meuse, and in drinking water, followed by in vivo exposure experiments of male fish to Rhine and Meuse water. Both studies showed that levels of estrogenic activity were much higher in the Meuse than in the Rhine, which correspondingly led to significant vitellogenin production in only the male fish exposed to Meuse water. No estrogenic activity was found in drinking water.
As the estrogens are not the only hormones of which there may be mimicking activity in environmental water samples, attention is now turned to other types of hormone activities, as well. In collaboration with the association of Rhine Water

Works (RIWA-Rijn), thyroid activity was measured in three surface waters with a thyroid transport protein binding assay using a surface plasmon resonance based biosensor (Biacore 3000) at RIKILT Institute of Food Safety. Significant protein binding activity was found in these samples with this assay. Additionally, five different endocrine activities (estrogenic, androgenic, progestagenic, thyroid and glucocorticoid) are currently being measured simultaneously in various water samples, using the newly available battery of CALUX assays.

106 DEVELOPMENT OF AN AMPHIBIAN LIFE CYCLE ASSAY FOR ENDOCRINE DISRUPTOR TESTING.

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In 1998, the EPA outlined a plan for the Endocrine Disruptor Screening Program (EDSP) that incorporated a two tier testing approach. This poster summarizes the approach and methods we've developed for an amphibian life cycle assay utilizing the Tropical Clawed frog (Xenopus tropicalis) that would be suitable as a second tier EDSP test. Endpoints were designed around three specific androgen- or estrogen-mediated processes: 1) differentiation of the gonads, 2) secondary sex characteristic development, and 3) reproductive maturation. Screening for sex-linked amplified fragment length polymorphisms was carried out in order to develop a method for genotyping sex in X. tropicalis. Polymorphisms located closest to the sex locus (<2% recombination) were used to develop a PCR-based method for genetic sex determination. Expression of sexually-dimorphic genes in the gonads was measured by quantitative rtPCR in order to determine phenotypic sex as well as identify intersex in newly-metamorphed tadpoles. Methods for evaluating nuptial pad formation, an androgen sensitive tissue, were developed. An ELISA for measuring vitellogenin, a biomarker of estrogenicity, was developed. As a surrogate for measuring fecundity in this organism, oocyte and sperm counting methods are utilized in adult frogs. Use of these endpoints are illustrated from exposures to model endocrine disruptors, specifically 17α ethynylestradiol, 17β trenbolone, and fadrozole. Sex reversal and intersexuality was identified in tadpoles exposed to ethynylestradiol and fadrozole. Premature nuptial pad formation and vitellogenin induction were both observed immediately after metamorphosis following exposure to trenbolone and ethynylestradiol, respectively. Oocyte and sperm counts were used in control baseline data to establish reproductive maturation when raised under test conditions. These methods are combined in a proposed full life cycle amphibian development, growth and reproduction test that would be used as part of the battery of tests in the EDSP.

107 USE OF A NOVEL MICROPLATE READER-BASED HIGH-THROUGHPUT ESTROGEN RECEPTOR BINDING ASSAY IN PREDICTING COMBINATION EFFECTS.

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Coumestrol is a well-known ligand for the estrogen receptor (ER). The compound itself is fluorescent, and its fluorescence intensity at 408 nm increases upon binding to the ER. We previously published a novel binding assay in 96-well plate format for estrogenic compounds, based on the competition between fluorescent coumestrol and estrogenic compounds for binding to the ligand binding domain (LBD) of the ER-alpha (1). Competitive binding curves for six well-known estrogenic compounds, 17-estradiol (E2), ethinylestradiol, 4-nonylphenol, 4-octylphenol, genistein and bisphenol A, were constructed. EC50 values and relative potencies (compared to E2) of the compounds were determined. A chemical mixture was created by combining six test chemicals at ratios proportional to their potency. Based on these ratios and the concentration-response data for each individual agent, predictions of mixture effects were calculated. In the 6 component EC50 mixture experiment, concentration addition (CA) approach slightly underestimated the EC50 of the mixture. Explanations for this observation were discussed. In view of the fact that in environmental risk analysis, assessments are typically made using orders of magnitude, the observed deviations between predictions and observations can be considered negligible. Results led us to the overall conclusion that CA predicts the joint action of estrogens also reasonably well in the present receptor binding assay.

1. Gurer-Orhan, H.; Kool, J.; Vermeulen, N.P.E.; Meerman, J.H.N. A novel microplate reader-based high-throughput assay for estrogen binding. Int. J. Environ. Anal. Chem. 2005, 85, 149-161.

108 *IN VITRO* EFFECTS OF ESTRADIOL PRESENT IN COW'S MILK DETERMINED WITH MICROARRAYS AND A CO-CULTURE SYSTEM OF CACO-2 AND MCF-7 CELLS.

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Dietary exposure to estrogens, their metabolites and other estrogenic compounds has been associated with fertility problems and hormone dependent cancers.This study focuses on estradiol (E2) levels in cow's milk and its effects on biologically relevant tissues. HPLC-MS/MS analysis of commercially available cow's milk showed that E2 concentration was around 0.04 nM. A similar concentration of E2 induced no biochemical effects on cell proliferation or cell cycle in colon (Caco-2) cells and only minor induction of cell proliferation in MCF-7 (breast cancer) cells. After 24hrs incubation, microarrays were done, confirming the minor effects on Caco-2 cells, while showing a significant time dependent upregulation of gene categories related to cell cycle, cell proliferation, mitosis, nuclear division and DNA replication in MCF-7 cells after exposure to these E2 levels was observed. A total of 412 genes was found to be up or downregulated in this cell line (> 1.5 or < -1.5 fold). A number of upregulated genes related to the etiology of breastcancer were also confirmed with RT-PCR. These genes included among others BRCA1, CKD6, c-Jun, PIK3R3, E2F1, FANCD2. Thus, realistic concentrations of E2 in milk can potentially induce a biological effect related to estrogen dependent tumors. To develop a more realistic in vitro model, mimicking transport across the small intestine and also including biotransformation properties, we made a physiologically based co-culture system of Caco-2 cells on a semi-permeable membrane with an underlying culture of MCF-7 cells. This co-culture system is stable for longer time periods and is now used for testing various estrogenic compounds related to dairy consumption. Relevant endpoints are expression of CYP1A1 and 1B1 in CaCO-2 and MCF-7 cells for estrogen metabolism, while pS2 expression in the MCF-7 cells is used as a measurement for estrogenic activity.

109 COMPARISON OF ACRYLONITRILE-INDUCED OXIDATIVE DNA DAMAGE IN CULTURED RAT AND HUMAN ASTROCYTES.

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Acrylonitrile, widely used in the manufacturing of plastics, acrylic fibers, and synthetic rubber, resulted in a dose-related increase in gliomas in rats following chronic exposure. Previous studies have shown that that the mechanism for the induction of brain tumors by acrylonitrile involves the induction of oxidative stress. The present study compared the ability of acrylonitrile to induce both direct and oxidative DNA damage in cultured rat astrocytes and human astrocytes using alkaline (direct DNA damage) and formamidopyrimidine DNA glycosylase modified (oxidative DNA damage) Comet assay. No increase in direct DNA damage was observed in either cell type following exposure to sublethal concentrations of acrylonitrile (0-1.0 mM) for 24 hours. Acrylonitrile induced a concentration-related increase in oxidative DNA damage in rat astrocytes (up to 2.5 fold over control), but not in human astrocytes after 24-hr exposure. Glutathione (GSH) conjugation is a major metabolic pathway of acrylonitrile and may be involved in the oxidative stress induction by acrylonitrile. Measurement of GSH showed that human astrocytes had a higher basal level of GSH than rat astrocytes (~ 55 vs ~ 40 nmol/mg protein). Twenty-four hour treatment with 0.1 mM buthionine-sulfoximine resulted in over 90% depletion of GSH in both cell types (92% in rat astrocytes and 97% in human astrocytes). The depletion of GSH increased ACN-induced oxidative DNA damage in a concentration-dependent manner (ranging from 22 to 46%) in rat astrocytes. Similarly, an increase in oxidative DNA damage was observed in GSH depleted human astrocytes (up to 2.6 fold over control). On the other hand, treatment with L-2-oxothiazolidine-4-carboxylic acid (2.5 mM), a GSH biosynthesis precursor, resulted in a 58% increase in glutathione level and attenuated ACN-induced oxidative DNA damage (7-47%). The results of this study suggest that human astrocytes are more resistant to acrylonitrile-induced oxidative DNA damage compared to rat astrocytes, and that GSH is a contributing factor in the induction of oxidative DNA damage.

110 GENDER DISPARITY IN EARLY OCHRATOXIN A MEDIATED RENAL TOXICITY.

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Ochratoxin A (OTA) is a powerful nephrocarcinogen in male rats, probably acting by a mechanism leading to oxidative DNA damage. However, the causal factors for sex-differences associated with OTA-mediated carcinogenicity remain unclear. This

study investigated whether OTA administration affects the antioxidative defence mechanisms and/or cell proliferation in a gender-specific fashion. Male and female Eker rats (heterozygous for a mutation in the Tsc2 tumor suppressor gene) and corresponding wildtype (WT) rats were gavaged daily with 210µg OTA/kg BW for up to 14 days. Subsequent examination of renal pathology, cell proliferation (via PCNA immunohistochemistry), and expression analysis of genes involved in oxidative stress via real-time PCR, demonstrated that: (a) OTA led to a higher increase in cell proliferation in male than in female Eker and WT rats, when compared with the corresponding controls, (b) OTA induced a higher number of basophilic atypical tubules in male than in female Eker rats, while OTA-treated WT rats did not show significant changes versus their corresponding controls, and (c) In female, but not male Eker and WT rats, OTA treatment led to an increased gene expression of the DNA repair gene 8-oxoguanine DNA glycosylase (OGG1). Furthermore, male but not female OTA treated rats showed a decreased expression of the antioxidative enzyme catalase (CAT). Conclusion: Male rats may be more susceptible to reactive oxygen species due to the down-regulation of CAT. Conversely, female rats may have better protection from OTA-mediated oxidative stress due to the up-regulation of OGG1. The gender specific regulation of critical enzymes protecting against oxidative stress, support the hypothesis of reactive oxygen species induced tumor initiation in male rats. Moreover, enhanced oxidative stress may lead to higher regenerative cell proliferation and thus tumor promotion in male than in female rats. Indeed, OTA administration in Eker rats, already carrying a mutation in the Tsc2 tumor suppressor gene, leads to enhanced propagation of pre-neoplastic lesions in both genders.

111 EXPRESSION OF CYCLINS AND CYCLIN-DEPENDENT KINASES IN RAT SPLEEN FOLLOWING ANILINE EXPOSURE.

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Aniline exposure is associated with selective toxicity to the spleen leading to splenomegaly, hyperplasia, and a variety of sarcomas of the spleen on chronic exposure. In earlier studies, we have shown that aniline exposure leads to iron-overload, oxidative stress and activation of redox-sensitive transcription factors, which could regulate various genes leading to a tumorigenic response in the spleen. The present study was focused on evaluating the expression of cell cycle proteins (cyclins) and cyclin-dependent kinases (CDKs) in the spleen, in an experimental condition preceding a tumorigenic response. Male SD rats were treated with aniline (0.5 mmol/kg/day via drinking water) for 30 days (controls received drinking water only), and splenocyte proliferation and expression of cell cycle related proteins were measured. Aniline treatment resulted in significant increases in splenocyte proliferation, determined by MTT assay and flow cytometry, compared to the controls. Western blot analysis of splenocyte proteins from aniline-treated rats showed significantly increased expression of cyclin D2, cyclin D3, cyclin E and cyclin A compared to the controls. The over-expression of these cyclins was associated with significant increases in the expression of CDK2, CDK4, CDK6, CDC2 and a significant decrease in the expression of check point protein p27. Our data suggest that changes in the expression of cyclins and CDKs could be critical in cell proliferation, and may contribute to aniline-induced tumorigenic response in the spleen. (supported by NIH ES06476)

112 PROPICONAZOLE-INDUCED CARCINOGENESIS: ROLE OF OXIDATIVE STRESS.

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Propiconazole is a systemic foliar fungicide with a broad range of activity. Rodents fed with propiconazole at high dose resulted in diminished body weight, increased liver weight of adults and pups, and eventually liver carcinogenesis. In order to unravel the toxic processes involved in carcinogenesis as well as to obtain insight into molecular basis and mode of action of propiconazole-induced tumorigenesis we have performed a genomic analysis of propiconazole-treated mouse livers using cDNA arrays. Some important differentially expressed genes and pathways involved in oxidative stress have been identified, suggesting the roles of oxidative stress in propiconazole-induced carcinogenesis. To get a better understanding of oxidative-stress mediated toxicity or carcinogenesis, we have recently developed an integrated proteomic technology platform for analysis of oxidative stress status, antioxidant ability, protein oxidation and identification of biomarkers of oxidative stress during propiconazole-induced carcinogenesis. As the first step, we performed systematic assays for the measurement of antioxidant enzymatic activities of superoxide dismutase, catalase, glutathione s-transferase, GSH/GSSG ratio, total thiol and carbonyl content of proteins in mouse liver tissues treated with propiconazole for four days. A significant decrease in GSH/GSSG ratio, and an increase in carbonyl content and glutathione s-transferase activity in propiconazole-treated mouse livers were observed. To our knowledge, these results may be the first and direct proteomic evidence about involvement and roles of oxidative stress in propiconazole-induced carcinogenesis. In addition, oxidative stress activated-carcinogenesis pathways and toxic mechanisms of propiconazole are under investigation.
This abstract does not necessarily reflect EPA policy.

113 MECHANISTIC STUDY ON HEPATOCARCINOGENESIS OF PIPERONYL BUTOXIDE IN MICE.

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Our previous studies demonstrated that several reactive oxygen species (ROS)-related genes are significantly up-regulated in the liver of male mice administered 0.6% piperonyl butoxide (PBO), a-[2-(2-butoxyethoxy)ethoxy]-4,5-methylenedioxy-2-propyltoluene, for 6 weeks. In order to clarify the possible mechanism of non-genotoxic hepatocarcinogenesis induced by PBO, male ICR mice were subjected to a two-third partial hepatectomy. Twenty four hours later, the mice received an i.p. injection of N-diethylnitrosamine (DEN) to initiate hepatocarcinogenesis. One week later, they received 0.6 or 0 % PBO-containing diet for 8 weeks. After sacrifice at the termination of this experiment, the livers were histopathologically examined and subjected to gene expression analyses using cDNA microarray and real-time RT-PCR. Significant increases in gamma-glutamyl transpeptidase (GGT) and proliferating cell nuclear antigen (PCNA) positive cells were observed in the DEN + PBO group, as compared to the DEN alone group. In the microarray analysis, genes related to metabolism such as cytochrome P450 (Cyp) 1A1, Cyp2b9 and Cyp2b10, and metabolic stress related genes such as NADPH-cytochrome P450 oxidoreductase (Por), NAD(P)H dehydrogenase, quinine 1(Nqo1), multidrug resistance associated protein 2 (Abcc2), Abcc3 and Abcc4 were up-regulated in mice of the DEN + PBO group. Fluctuations of these genes were quantitatively confirmed by the real-time RT-PCR in this group. Additional real-time RT-PCR analysis revealed that the genes related to oncogene of c-jun, c-myc and stress response-inducible transcription factor ATF3 were significantly increased in this group. These results suggest the possibility that PBO has the potential to induce oxidative stress, including oxidative DNA damage, and the increased cell proliferation function results in the induction of hepatocellular tumors in mice.

114 PROTEIN DAMAGE FROM OXIDATIVE STRESS ACCUMULATES IN LUNGS OF MICE EXPOSED TO ELECTROPHILIC TUMOR PROMOTERS DERIVED FROM BHT.

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The food additive butylated hydroxytoluene (BHT) enhances tumorigenesis in the lungs of mice treated with a single dose of an initiator followed by multiple doses of BHT, and several lines of evidence implicate inflammation as a causative factor [A.K. Bauer et al., Exp. Lung Res. (2001) 27, 197-216.]. A general relationship between oxidative stress and tumor promotion is well established. BHT is metabolized by P450 in mouse lung to produce two para-quinone methides that are electrophilic and form covalent adducts to proteins including peroxiredoxin 6, Cu,Zn-SOD, and carbonyl reductase [B.W. Meier et al., Chem. Res. Toxicol. (2007) 20, 199-207]. Each of these protective enzymes is inhibited by alkylation resulting in increased levels of hydrogen peroxide, superoxide, and products from lipid peroxidation. In the present work, we examined the results of sustained oxidative stress from multiple BHT injections, i.e. the protocol used for tumor promotion, by assessing the accumulation of protein damage over time. Cytosolic fractions from the lungs of mice receiving either a single injection or four weekly injections of BHT were compared with cytosols from untreated mice. We measured protein oxidation, nitrosation, and adduction by reactive aldehydes derived from lipid peroxidation with western blotting and mass spectrometry. The data show that there are substantial increases in protein damage by reactive oxygen species and aldehydes including 4-oxonon-2-enal in lungs that correlate with BHT exposure and provide additional support for oxidative stress as an important component of BHT-induced lung tumor promotion in mice. (Supported by NIH grant CA04128)

115 ALTERATION OF AIRWAY EPITHELIAL HOMEOSTASIS BY VANADIUM PENTOXIDE (VO).

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Exposure to environmental pollutants has been linked to various airway diseases such as COPD, Asthma, and Lung cancer. Vanadium is one of the major components of metal-rich combustion pollutant-residue oil fly ash (ROFA), and

Vanadium Pentoxide (VO) has been widely used as a catalyst in industrial production. Inhalation of ROFA or vanadium vapor has been shown to cause COPD-like symptoms including mucous over-production, airway obstruction, inflammation etc. Recent NTP study suggests that chronic VO exposure may cause lung cancer in different rodent models. To understand the mechanistic basis of VO-induced airway remodeling and carcinogenesis, we have examined VO-induced alterations of airway epithelia in both in vitro and in vivo models. Using combinatorial approaches, we have found that VO induced severe airway inflammation, mucous cell metaplasia, lung injury and repair. At molecularly level, VO significantly increased epithelial cytokine and growth factor production, and persistently activated proliferation/surviving pathways including ErbBs, PI3K-AKT, and MAPK. In addition, VO, instead of vanadate, induced substantial amount of Reactive Oxygen Species (ROS) generation in both intra-cellular and extra-cellular compartments, which may further caused the oxidation of cellular components and DNA strand break. On the other hand, VO transcriptionally repressed almost all major components of the repair machinery for DNA strand break. Thus, the carcinogenic activity of VO is likely to depend on its action in promoting DNA mutation and enhancing proliferation and survival of malignant cells.

116 LUNG TUMOR PROMOTION BY CURCUMIN.

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Curcumin has been shown to exhibit anti-tumor activity in a variety of cell culture and *in vivo* animal models and is currently being tested in clinical trials as a chemopreventive agent for colon cancer. We tested the chemopreventive activity of curcumin in a transgenic mouse model that expresses the human Ki-*ras*^{G12C} allele in a doxycycline (DOX) inducible and lung specific manner. Treatment of DOX-induced mice with a curcumin enriched diet (4000 ppm) increased tumor multiplicity±SE (from 24.3±0.2 to 36.3±0.9) and progression to later stage lesions, similar to animals that had been co-treated with DOX and the known lung tumor promoter butylated hydroxytoluene (BHT). Sections of lungs examined microscopically demonstrated increased adenoma (AD), large atypical AD, and adenocarcinoma development in the DOX/curcumin (66% of lesions) and DOX/BHT/curcumin (49% of lesions) treated groups compared to DOX only treated mice, where only 19% of lesions were classified as ADs. Semi-quantitative immunohistochemistry demonstrated that increased tumor multiplicity and progression was associated with statistically significant alterations in apoptotic signaling pathways – as demonstrated by decreased expression of *p-p53*, p19, caspase-3 activity and increased expression of Ki-67 - and oxidative stress - as demonstrated by increased levels of pNF-κβ, iNOS, and staining for 2,4-dinitrophenylhydrazone, a marker for increased production of reactive oxygen species. Although curcumin and BHT exhibited somewhat different patterns of alterations in cell signaling pathways, when co-administered the two agents worked synergistically to enhance proliferative and survival signals in the bitransgenic mice. These results suggest that curcumin may exhibit organ-specific effects on early neoplastic cells and that ongoing clinical trials may need to exclude smokers in chemopreventive trials of curcumin at other organ sites.
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117 CC10 mRNA AND PROTEIN EXPRESSION IN CLARA CELLS OF CD-1 MICE FOLLOWING EXPOSURE TO STYRENE OR ITS METABOLITES STYRENE OXIDE OR 4-VINYLPHENOL.

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Styrene, used extensively in manufacturing, has adverse effects on rodent lung. Clara cells, the primary site of activity in the lung for styrene metabolism and its acute effects, are thought to be involved in the formation of mouse lung tumors following chronic styrene exposure. Clara cells produce CC10, an antioxidant and anti-inflammatory that may play a protective role in tumorigenesis, with decreases in CC10 possibly indicating preneoplastic changes. CC10 mRNA expression in Clara cells isolated from CD-1 mice showed no significant changes 3 hrs after in vitro treatment with styrene or styrene oxide (racemic, R-, S-). Significant decreases were seen after 4-vinylphenol treatment, but these results were somewhat negated by a decrease due to the carrier propylene glycol. When CC10 mRNA levels were measured after in vivo administration, there was a decrease with styrene oxide and 4-vinylphenol after 3 and 12 hrs, yet increases after styrene administration at both time points. CC10 protein levels increased following treatment with styrene metabolites after 3 hrs, with some levels declining after 12 hrs. CC10 protein levels, however, showed no change from controls after styrene. SPA protein expression changed very little following 3 and 12 hr treatments with styrene or styrene oxide. Decreases were observed with 4-vinylphenol at both time points and propylene glycol at 12 hrs. When older mice (11 months) were treated in vivo with styrene and racemic styrene oxide decreases in CC10 mRNA expression were seen 12 hrs after administration. For styrene, this effect was opposite to that seen in younger mice. CC10 protein levels in older mice showed no significant changes. These studies show that acute changes in lung protein and mRNA expression do occur following in vivo treatment with styrene or its metabolites. These changes following acute lung injury may be indicators of inflammatory processes and preneoplastic changes. (This work was supported in part by a grant from the Styrene Information and Research Center.)

118 EFFECTS OF ESTRADIOL ON TCDD-INDUCED OXIDATIVE STRESS IN LIVER CELLS.

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2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has been classified as a human carcinogen, but the mechanisms of its carcinogenic effects, e.g. in rodent liver, are not fully understood. Most if not all of its effects such as transcription of cytochromes P450 (CYP) 1A1, 1A2, and 1B1, oxidative stress and tumor promotion are thought to be mediated by the arylhydrocarbon receptor (AhR). Previous studies have shown that TCDD treatment leads to an estrogen-dependent increase in 8-oxo-dG in hepatic DNA of female rats. It has been postulated that the 17β-estradiol (E2) metabolite 4-hydroxyestradiol formed by CYP1B1, could undergo redox cycling and thus generate DNA damaging reactive oxygen species (ROS). The corresponding quinone has been reported to cause DNA damage. In our study, TCDD (1 and 10 nM) treatment of rat hepatocytes and rat H4IIE hepatoma cells, but not human HepG2 cells, led to a significant increase in ROS formation which was not affected by addition of E2 (100 nM). However, E2 alone resulted in a significant increase in ROS formation in rat hepatocytes. Real-time RT-PCR analysis showed that TCDD but not E2 induced CYP1B1 in the hepatoma cell lines HepG2, H4IIE, and in rat primary hepatocytes from male and female rats. CYP1B1 mRNA was expressed at higher levels in rat hepatocytes. Alkaline Comet assay revealed that neither TCDD nor E2 resulted in a significant increase in the level of DNA fragmentation compared to controls. The presence of oxidative DNA damage using a Comet assay including a formamidopyrimidine glycosylase (fpg) digestion step will be further investigated. In addition, we used DNA microarray technique in order to elucidate global gene expression profiling of HepG2 cell line after TCDD treatment.

119 CHEMOPREVENTION OF GASTROESOPHAGEAL REFLUX-INDUCED ESOPHAGEAL ADENOCACINOMA BY α-TOCOPHEROL, N-ACETELCYSTEINE, AND OMEPRAZOLE IN RAT MODEL.

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Esophagogastroduodenal anastomosis (EGDA) will induce esophageal adenocarcinoma (EAC) in rats. We previously demonstrated that oxidative stress is a critical element in the progression of the disease. The present study investigated the possible chemopreventive effects of two antioxidants, α-tocopherol and N-acetylcysteine (NAC) independently and in combination. We also looked the chemopreventive effect of therapeutic antacid agent, omeprazole in this study. Two weeks following EGDA, rats were begun on the diets containing α-tocopherol at 389ppm or 778ppm (5 and 10 folds of the basal dietary level), NAC at 500ppm or 1000ppm, their combination (389ppm α-tocopherol plus 500ppm NAC), or 1400ppm omeprazole. All animals were sacrificed 40 weeks after surgery and the esophagus was removed for histopathological examination. α-tocopherol decreased the incidence of EAC in a dose-dependent manner (P=0.03) and 778ppm α-tocopherol significantly reduced the incidence of EAC from 84% (26/31) to 59% (16/27) (P=0.04). The supplement of α-tocopherol increased the plasma concentration of α-tocopherol from 30.7±10.9μmol/L in the control group to 55.2±17.7μmol/L of the 778ppm α-tocopherol group. NAC at 500ppm and 1000ppm appeared to decrease EAC incidence, but the effect was not statistically significant. However, the combination of α-tocopherol and NAC significantly reduced the incidence of EAC to 55% (15/27) (P=0.02). Omeprazole showed a slight but insignificant chemopreventive effect at the dose given. In conclusion, we found that α-tocopherol can prevent the development of EAC in our EGDA model with rats, and stronger effects can be achieved when used in combination with NAC (Supported by NIH grant CA75683).

120 CHARACTERIZATION OF GLUTATHIONE *S*-TRANSFERASE ALPHA 4 (*GSTA4*) AS A MODIFIER OF TUMOR PROMOTION SUSCEPTIBILITY IN THE MOUSE TWO-STAGE SKIN CARCINOGENESIS MODEL.

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Genetic differences in susceptibility to two-stage skin carcinogenesis in the mouse have been well described. Using genetic crosses of sensitive DBA/2 mice with relatively resistant C57BL/6 mice, loci that modify 12-*O*-tetradecanoylphorbol-13-acetate (TPA) tumor promotion response have been mapped to chromosomes 1, 2, 9, and 19. Recent studies suggest that glutathione *S*-transferase alpha 4 (*Gsta4*), a gene that maps within the *Psl1.2* locus on chromosome 9, may be a good candidate for a gene that modifies susceptibility to skin tumor promotion. To investigate this hypothesis, we have confirmed that *Gsta4* mRNA and protein are dramatically elevated in the epidermis of C57BL/6 versus DBA/2 mice following TPA treatment. Epidermal lysates from TPA-treated C57BL/6 mice also had elevated *Gsta4* enzyme activity toward the model substrate, 4-hydroxynonenal, compared with lysates from TPA-treated DBA/2 mice. Furthermore, *Gsta4* was induced in C57BL/6 epidermis following treatment with other mechanistically diverse tumor promoting agents (chrysarobin and okadaic acid) suggesting that this response may play a generalized role during tumor promotion. Mice lacking the *Gsta4* gene exhibited mildly enhanced epidermal proliferative response to TPA as compared with wild-type C57BL/6 mice. In two-stage skin carcinogenesis studies using DMBA as the initiating agent, *Gsta4* deficient mice developed skin tumors with a shorter latency and in greater number compared to wild-type mice when TPA (3.4 nmol) was used as the promoting agent. Therefore, *Gsta4* induction in C57BL/6 mice may explain the protective effect of *Psl1.2* on TPA skin tumor promotion susceptibility.

121 THE ROLE OF KUPFFER CELLS IN ETHANOL-INDUCED HEPATOCELLULAR GROWTH.

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Chronic ethanol consumption has been linked to an increased risk of hepatocellular cancer in humans and rodents, however, the mechanism(s) involved have not been resolved. Research examining the mechanisms leading to ethanol-induced hepatotoxicity has shown that Kupffer cell activation, resulting from increased endotoxin levels, appear to mediate hepatocyte damage and necrosis. Since Kupffer cells produce bioactive products such as reactive oxygen species and cytokines following their activation, substances that can also elicit growth stimulatory effects, we questioned whether the Kupffer cell participates in increased hepatocellular growth following ethanol exposure. To identify doses of ethanol that increased hepatocellular growth and were not hepatotoxic, a dose response study for ethanol (0 – 5% ethanol (w/v) in liquid diet) was conducted in male C57Bl mice. Exposure to ethanol for 7 days increased hepatic DNA synthesis (~2-fold over control) at concentrations of 2% and 3% ethanol (w/v). Higher ethanol doses (4% and 5%) failed to increase DNA synthesis, but increased hepatotoxicity as evidenced by increased serum ALT and AST. Ethanol doses between 1 – 3% ethanol were without toxicity. We next examined the role of the Kupffer cell on ethanol-induced DNA synthesis by exposing mice to 3% ethanol in the presence and absence of Kupffer cell depletion (using clodronate liposomes). Clodronate liposome treatment resulted in >90% depletion of Kupffer cells, assessed using F4/80 immunohistochemistry. 3% ethanol produced the expected increase (~2-fold) in DNA synthesis relative to control levels. However, Kupffer cell depletion reduced ethanol-induced hepatic DNA synthesis to below control levels (~1.5% compared to ~2.0% in controls). These studies support that Kupffer cells participate in the induction of DNA synthesis and the hepatocellular growth response seen following ethanol exposure, and have identified a model system in which the mechanisms involved in ethanol-induced cell growth can be further defined.

122 LONG-TERM, LOW-LEVEL EXPOSURE OF UROTSA CELLS TO MONOMETHYLARSONOUS ACID PRODUCES PROTEIN CARBONYL MODIFICATIONS AND OXIDATIVE DAMAGE TO DNA.

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Chronic low level exposures of monomethylarsonous acid (MMAIII) have been associated with the malignant transformation of the human bladder urothelium. Evidence has shown MMAIII to induce the formation of reactive oxygen species (ROS) leading to DNA damage, as well as, the oxidative modification of proteins. Protein oxidation and DNA damage associated with oxidative stress as a result of ROS have a major impact on carcinogenesis. The non-tumorigenic human urothelial cell line, UROtsa, following continuous exposure to 0.05 µM MMAIII for up to 52 wk (MSC52) was examined for the detection of MMAIII/ROS oxidation of proteins and DNA damage. UROtsa cells treated with 0.05 µM MMAIII for 12, 24, or 52 wk were analyzed by western blot for protein carbonyl modifications as a marker for protein oxidative status, which was immunodetected using 2, 4-dinitrophenylhydrazine. Following chronic MMAIII exposure, protein carbonyl modification increased with duration of exposure, with the highest protein carbonyl levels following 52 wk exposure. These findings correlate to previous studies demonstrating increased protein carbonyl levels in patients with bladder cancer. As there is limited information concerning the genotoxic potential of chronic low level exposure to MMAIII, the MSC52 cell line was used to investigate the potential DNA damage through the assessment of single strand breaks using the single-cell gel electrophoresis "comet assay" technique. Following continuous exposure of 0.05 µM MMAIII for 12, 24, 36, or 52 wk, there is a time-dependent increase in comet tail length and subsequent increase in DNA single strand breaks when compared to untreated UROtsa cells. This data opens the possibility of an association between chronic low-level arsenical exposure and genotoxic insult/protein modification as a result of ROS induction, and the overall contribution to bladder carcinogenicity. (NIH ES04040, ES06694, ES07091)

123 CHRONIC EXPOSURE TO MONOMETHYLARSONOUS ACID INDUCES CHANGES IN HUMAN UROTSA CELLS CONSISTENT WITH UROTHELIAL TUMORIGENESIS.

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Urothelial tumorigenesis has been characterized as a divergent pathway with unique genetic and epigenetic alterations for both the non-invasive and the invasive pathway. Previous studies have shown that chronic treatment with monomethylarsonous acid [MMA(III)] induces cancer-like properties in UROtsa cells including hyperplasia, growth in soft agar, and tumor development when transplanted in SCID mice. This study characterizes the spectrum of changes that occur as a result of chronic MMA(III) treatment as it relates to known characteristics of bladder carcinogenesis, including aberrant expression of E-cadherin, N-cadherin, matrix metalloproteinases, cyclooxygenase 2, and ras. UROtsa cells were treated with 50 nM MMA(III) daily for 12 months, and protein isolates were collected at the end of each month of treatment. Decreased E-Cadherin and increased N-Cadherin expression correlate with an invasive phenotype. Western Blot analysis showed a decrease in E-cadherin expression by month 7 of MMA(III) treatment. Gene array data confirms that CDH1 mRNA, the promoter region for E-cadherin, is down regulated by <0.5 in samples treated for 5 months, and that CDH2 mRNA, the N-cadherin promoter region, is increased six fold at month 12. Over-expression of MMPs are further indicative of an invasive phenotype, and MT-MMP1 mRNA is increased 3-fold by month 12. COX-2, a protein found elevated in invasive urothelial carcinomas was also found to be elevated in both the 6 and 12 month MMA(III) treated UROtsa cells. Over-expression of the ras oncogene, however, is specific to low-grade non-invasive papillary carcinomas. Immunoprecipitation data showed a gradual induction of ras up to month 5 of treatment, at which point ras expression began to decline. Chronic exposure to MMA(III), therefore, models the development of urothelial tumorigenesis, showing characteristics of non-invasive papillary tumors in the early months of MMA(III) treatment and later advancing to the invasive high-grade stage with prolonged exposure beyond 5 months of treatment. (NIH ES04040, ES06694, ES07091)

124 PROTECTIVE EFFECTS OF BLACK TEA ON OXIDATIVE DAMAGE IN HUMANS.

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A double-blinded, placebo-controlled study in 85 healthy volunteers (smokers and nonsmokers) was performed to assess the effect of black tea consumption on oxidative damage. Individuals were given tea (2.4g/day) for two weeks subsequent to placebo treatment. Single cell gel electrophoresis (Comet) was used to measure DNA strand breaks in peripheral white blood cells (WBCs). WBC 8-OHdG was also determined. In addition, plasma antioxidants (vitamin E, Total Antioxidant Capacity (TAC)) and lipid peroxidation (malondialdehyde, MDA) were determined. The basal level of Comet was ~ 20% greater in smokers than nonsmokers. Males showed a higher level of Comet (~15%) compared to respective females. Consumption of black tea reduced DNA strand breaks in all participants. The reduction was greater in males compared with females (22% vs. 11%), and in smokers compared to nonsmokers (27% vs. 16%). The protective effect of black tea Comet was greater in male nonsmokers compared with female nonsmokers (21% vs. 2 %), whereas in male or female smokers, black tea reduced DNA strand breaks to a similar extent (~25%). Also, tea significantly reduced WBC 8-OHdG (17%) in both males and females but showed a greater reduction in smokers compared

with non-smokers (27% vs. 9%), with a greater effect seen in male smokers compared to male nonsmokers (28% vs. 8%). In contrast, consumption of black tea significantly increased the plasma vitamin E levels. This increase was greater in males (17%), smokers (19%) and male smokers (23%) compared with females, nonsmokers and male nonsmokers, respectively. Plasma TAC and MDA levels were not significantly different after tea consumption. Furthermore, plasma vitamin E levels were negatively correlated with Comet and plasma MDA. These results demonstrate that black tea is protective against DNA damage including oxidative DNA damage in humans, and suggest a greater protection in smokers. Supported in part by NIH CA100908.

125 KINETICS OF SUBCUTANEOUS EXPOSURE TO SUB-LETHAL VX, GF AND GB DOSES IN GUINEA PIG.

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Mathematical models of absorption, distribution and elimination of chemical warfare nerve agents are used to improve toxicity estimates, extrapolate to humans and predict response to a range of exposures. These studies compared toxicity and kinetic data following sc exposure to different nerve agents in male guinea pigs. Animals were given single sub-lethal sc doses of VX, GB or GF (0.1 and 0.4 x LD_{50}). Blood levels of AChE and BuChE, and regenerated VX (VX-G), GB (rGB) or GF (rGF), were evaluated at multiple time points after exposure. In plasma, for each exposure of VX, GF or GB, kinetic parameters such as area under the curve (AUC), maximum concentration (C_{max}), time to maximum concentration (t_{max}) and clearance (CL) were similar for each respective dose for rGB and rGF; however, values for these parameters except CL were different for VX-G (much lower AUC and C_{max} and much higher t_{max}). Furthermore, plasma half-life ($t^{1}/_{2}$) for rGF and VX-G was twice and four times as long, respectively, compared to rGB. In RBC, similar kinetic results occurred for G-agents except that clearance from RBC was higher for rGF than rGB and the $t^{1}/_{2}$ for 0.1 x LD50 GB dose was higher than other RBC $t^{1}/_{2}$ values. RBC AUC and C_{max} for VX-G were much less compared to G-agents; however, other RBC kinetic parameters for VX-G were fairly similar to those of the G-agents. For all agents t_{max} was higher for RBC than plasma, while reverse was true for Cmax. It appears that VX and GF have a greater inhibitory action upon guinea pig AChE activity, one that occurs earlier and persists longer, than does GB; however, both GF and GB had a greater inhibitory effect upon plasma BuChE than did VX. Although some kinetic parameters were similar for VX-G, rGB and rGF, differences did occur that were likely due to different physicochemical properties of the agents. Data will be utilized in physiologically based pharmacokinetic/pharmacodynamic model developed to simulate low-level nerve agent exposures and the results applied to risk assessment determinations.

126 POST-EXPOSURE PROTECTION AGAINST SOMAN-INDUCED LETHALITY WITHOUT CARBAMATE PRETREATMENT IN GUINEA PIGS.

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Acute exposure to soman (GD), an organophosphorus nerve agent, causes widespread cholinesterase inhibition which subsequently leads to seizures, convulsions, cardiorespiratory depression, and death. Currently, the modality adopted by the U.S. armed forces for managing GD poisoning involves carbamate (pyridostigmine, PYR) pretreatment followed by a post-exposure therapy consisting of atropine, an oxime and, if needed, diazepam. For military/civilian populations that are not enrolled in the PYR pretreatment program, however, the post-exposure component of this modality offers little, if any, protection against the toxic and lethal effects of nerve agents. To reduce the reliance/need for pretreatment, we have developed a post-exposure therapy mix that can protect against the lethal effects produced by a supra-lethal dose of GD (5xLD50, 130 μg/kg, sc) in unanesthetized guinea pigs. To permit a thorough efficacy evaluation, the animals were chronically instrumented to permit concurrent recordings of EEG, diaphragm and skeletal muscle EMG, integrated diaphragm EMG (τ=50 msec), ECGII and core temperature. The therapy mix, given within a min after GD, consisted of scopolamine (0.5 mg/kg, im), atropine methylnitrate (2 mg/kg, im), physostigmine (0.075 mg/kg, im), MMB4-(a bispyridinium oxime, 26.1 mg/kg, im), and phenobarbital (25 mg/kg, ip). Results showed that none of the animals tested exhibited seizures, convulsions or signs of anomalous cardiorespiratory activities or other debilitating symptoms associated with GD-induced cholinergic crisis. Only mild acute cholinergic effects (salivation, dystonia) and short-lasting phenobarbital-induced sedative effects were seen during the first 30 min after GD. The animals were typically asymptomatic within 60-100 min. All animals in this study survived the GD challenge 24 hr later. In conclusion, our findings indicated that the therapy mix used in this study was effective not only in antagonizing GD-induced lethality, but also in protecting the functional integrity of the CNS and cardiorespiratory system.

127 PHYSIOLOGICALLY-BASED MODELING OF NEUROTHERAPEUTICS.

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Antidotes for acute nerve agent (NA) exposures focus on rescue and/or the attenuation of seizures. Current NA prophylaxes involves multiple agents. These include a reversible acetyl cholinesterase (AChE) inhibitor (such as pyridostigmine bromide) to maintain a minimal fraction of AChE for regeneration; atropine sulfate to block muscarinic ACh receptors from excessive ACh buildup; and an oxime, to reactivate NA-inhibited AChE that isn't permanently bound or "aged". Despite these efforts to control the impact of ACh buildup, excessive glutamate release often results refractory seizures, requiring an anticonvulsant such as diazepam, within a narrow window of time. Seizures will ensue if the delicate balance between excitatory and inhibitory activity within the neuronal circuits remains disturbed. These pharmacodynamic interactions between nerve agents and specific countermeasures have not been quantitatively modeled previously. As an initial step toward this goal, an interactive physiologically-based pharmacokinetic and pharmacodynamic (PBPK/PD) model in the rat, describing the distribution of selected modulators of glutamaterigic and Gamma-Amino Butyric Acid (GABAregic) neurons and their respective effects on cerebral ACh, glutamate and GABA is proposed. A framework linking that neurotherapeutic model with existing mathematical models of AChE inhibition by nerve agents, via their interactive effects on excitatory and inhibitory neurotransmitters is presented. This approach may provide a means to extrapolate to humans for optimizing countermeasure therapies for specific nerve agent exposure scenarios.

128 SIMULATION OF VX EXPOSURE IN MULTIPLE SPECIES BY MEANS OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC/PHARMACODYNAMIC MODEL (PBPK/PD).

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Chemical warfare nerve agents (CWNA) are a potential threat to homeland security, as evidenced by the Aum Shinrikyo sect terrorist attacks with sarin (GB) in Matsumoto and in the Tokyo subway. To improve threat assessments for these types of incidents, a previously developed PBPK/PD model for GB was parameterized to simulate the kinetics and the dynamic affects of VX after subcutaneous, dermal or vapor inhalation in the guinea pig, rat, or Gottingen minipig. The PBPK/PD model was used to simulate agent exposure profiles from serial blood samples taken before, during, and after VX exposures for all species. Biomarkers of VX exposure simulated were the fluoride ion regenerated VX G-analog (VX-G) in red blood cells, blood plasma and tissues (Jakubowski 2006), as well as the classic inhibition of cholinesterases. Species differences in response were addressed with regards to the different PK profiles of VX. Previously published in vivo and in vitro estimates of VX hydrolysis were confirmed as a necessary model parameter difference from GB, which required as significant decrease to reproduce VX pharmacokinetics and pharmacodyanmics in all species. The blood concentration and urinary excretion of VX was used as a confirmation of the low in vivo metabolism of VX relative to other CWNAs, like GB and soman. This PBPK/PD simulation paradigm, validated with multiple data types in different species, provides the basis for predicting potential health effects if human populations are exposed to CWNAs.

129 A PHYSIOLOGICALLY-BASED DEPOSITION, CLEARANCE, AND SYSTEMIC BIOKINETIC MODEL OF INHALED BACILLUS ANTHRACIS SPORES.

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A biologically-based model of anthrax kinetics and dynamics was developed for multiple species to examine the mechanisms of spore deposition, clearance, and systemic kinetics on experimental outcomes in rabbits, and to perform cross-species extrapolation of disease response to humans. The typical path deposition model has compartments for the head, larynx, trachea, 13 bronchial airway generations, and 7 alveolar airway generations. The model describes deposition of inhaled Bacillus anthracis (BA) spores in each airway generation, phagocytosis by alveolar

macrophages, germination, clearance of vegetative bacteria to the tracheobronchial lymph nodes, and defeat of the macrophages via interruption of the MAP-kinase pathway by BA toxins followed by release to the systemic circulation. A systemic model of BA toxin for the whole animal was coupled with the deposition and clearance model to describe bacterial growth and the resulting levels of toxins in the body. The deposition/clearance predictions of the model agree well with the published data for the rabbit. Model predictions were compared to data collected on the number of spores and bacteria in broncho-alveolar lavage fluid and lung tissue. Prediction of lung spores following high-level exposure to groups of animals required incorporating inter-animal variability in measured exposure and breathing conditions to quantify initial deposited dose. The model is capable of simulating the number of the viable spores separately from the nonviable matter generated during aerosolization, and accounting for effects on clearance processes (e.g., macrophage recruitment, migration, and overload). The model was found to be sensitive to changes in breathing parameters, such as breath frequency and tidal volume, which affect the number of spores deposited in the alveolar region. A variability analysis of the human model was conducted to explore the implications of inter-individual differences on the health outcome of exposure to a diverse human population.

130 COMPARATIVE HEMATOTOXICITY OF HEXAHYDRO-1, 3, 5-TRINITRO-1, 3, 5-TRIAZINE (RDX) *VS.* MONO- AND TRI-NITROSO ENVIRONMENTAL DEGRADATION PRODUCTS MNX AND TNX.

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RDX, a contaminant of munitions manufacturing and artillery training sites, undergoes microbial nitroreduction to hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX), hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine (DNX), and hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX). MNX has been identified as the most potent acute toxicant (LD50 187mg/kg) of the degradation products of RDX (LD50 187 mg/kg). Based upon anemia that persisted 14 d after a single exposure to MNX (NOAEL 47mg/kg) and known reactivity of nitrogenous toxicants with hemoglobin, we hypothesized that hematotoxicity of these compounds is dependant on redox coupling of their N-nitro and/or N-nitroso groups with heme ferrous iron, with formation of methemoglobin, then anemia due to splenic sequestration of methemoglobin-containing erythrocytes. The objectives of the present investigation were to: 1) compare the effects of RDX, MNX and TNX on the formation of anemia and splenic hemosiderosis at both 7 and 14 d after a single, acute exposure, 2) determine whether RDX, MNX, or TNX could directly oxidize ferrous hemoglobin iron *in vitro* and 3) determine whether MNX was able to produce methemoglobinemia *in vivo*. Our results showed anemia due to RDX and MNX, but not TNX, as evidenced by decreased hemoglobin concentration 14 d, but not 7 d, after exposure. Splenic hemosiderosis, as determined by morphometric assessment of Prussian Blue-stained microscopic sections, was observed after MNX (14 d-NOAEL = 47mg/kg) and RDX (14 d-NOAEL = 24mg/kg; 7d-NOAEL = 12mg/kg), but not TNX. Methemoglobin formation was not observed in *in vitro* studies using rat blood incubated with up to 5mM RDX, MNX, or TNX nor *in vivo* with rats treated with 94 mg/kg MNX. In conclusion, our studies support a role of the N-nitro group in hematotoxicity of RDX and MNX, but suggest a mechanism independent of direct redox coupling with heme iron. Support: US Army Corps of Engineers.

131 KINEMATIC EVALUATION OF MOTOR IMPAIRMENT AND RECOVERY OF FUNCTION FOLLOWING SOMAN EXPOSURE IN A RAT MODEL.

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The chemical warfare nerve agent soman is an irreversible inhibitor of the enzyme acetylcholinesterase (AChE) and through the accumulation of acetylcholine (ACh) can lead to convulsions and respiratory failure. Animals that survive this insult display impaired motor and cognitive function, and severe brain pathology. Conventional treatments of nerve agent toxicity include an oxime to reactivate AChE, atropine to antagonize ACh at the muscarinic receptor, and an anticonvulsant to reduce seizure activity. However, these treatments do not provide complete protection against soman. In the current study, rats exposed to 1.0 LD50 soman were pretreated with the oxime HI-6, and then treated post-exposure with atropine and diazepam. Rat motor performance was evaluated through a series of kinematic trials on a balance beam and a clear walking platform for 3 weeks after soman exposure to evaluate degree of motor impairment and time course of recovery of function. Rats were given three separate tests, each measuring different aspects of gait. First, rats were given a kinematics test in which the limb joints were videotaped as the rat walked along a beam and then data analyzed via a computerized system. Next, the rats were videotaped from below as they walked along a clear plastic platform in a computerized gait analysis system. Last, the rats were given a pawprint trial along a beam, and the locations of the footprints were measured. Exposure to soman and conventional therapies induced severe motor impairment, with rats unable to maintain balance on the beam for days after exposure. Motor coordination recovered in the subsequent 3 weeks, evidenced by reduced latency to reach the goal box and improved consistency of paw placement within the RunwayScan and pawprint trials. These tests should prove useful in screening therapeutics for potential to provide additional functional protection against soman, above that provided by conventional therapies. Research was supported by the DTRA - JSTO, Medical S&T Division.

132 PREDICTABILITY AND RELIABILITY OF THE UP-AND-DOWN METHOD FOR DETERMINING LD50 VALUES FOR CHEMICALS WITH STEEP DOSE-RESPONSE CURVES: AN ANALYSIS OF INTRAVENOUS VX TOXICITY IN THE GOTTINGEN MINIPIG.

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O-Ethyl S-[2-(diisopropylamino)ethyl] methylphosphonothiolate (VX) is considered as the most toxic of the traditional nerve agents and therefore has an extremely steep dose-response curve. In the current studies sexually mature male Gottingen minipigs were exposed to liquid VX via intravenous (IV) injections. In the up-and-down study 8 animals were injected IV, 1 per concentration based on the animal's body weight, to calculate an LD50 value. A separate study was conducted using a classical paradigm. While the main objective of the classical study was not to determine an LD50 value we were able to do so. In the classical study a total of 32 Gottingen minipigs were used. Four animals were injected intravenously, at each of 8 different VX concentrations, based on the animal's body weight. Signs of nerve agent exposure were classified as lethal, severe, or moderate.
Maximum likelihood estimation using a probit model was used to calculate the median effective (severe effects) and lethal doses (LD50) for the IV VX exposures. Using the up-and-down test for intravenously injected VX, the LD50 was 11.8 μg/kg (9.7 – 14.5 μg/kg) and the ED50 (severe) was 6.6μg/kg (4.8 – 9.0 μg/kg). Using a classical study the LD50 was 11.8μg/kg (10.3 – 13.4 μg/kg) and the ED50 (severe) was 4.9μg/kg (3.7 – 6.6 μg/kg). The current findings support that the up-and-down method for determining LD50 values is a viable alternative to the classical toxicological study even for the most toxic of the traditional nerve agents, VX.

133 EFFICACY OF NOVEL PYRIDINIUM OXIMES TO REACTIVATE RAT BRAIN AND RED BLOOD CELL ACETYLCHOLINESTERASE INHIBITED BY ORGANOPHOSPHATES.

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Organophosphate (OP) poisoning resulting in the inhibition of acetylcholinesterase (AChE) traditionally has been treated with atropine sulfate, and an oxime, typically pralidoxime (2-PAM) or HI-6. One significant problem with the current oxime treatment is that the oximes display a very limited ability to cross the blood-brain barrier, resulting in little, if any, reactivation of AChE in the brain. A series of pyridinium oximes has been synthesized for this study that incorporate phenoxyalkyl moieties to increasee the lipophilicity to enhance the likelihood of crossing the blood brain barrier. The new oximes, along with 2-PAM, were evaluated *in vitro* to determine the ability of the oximes to reactivate AChE in both rat brain and erythrocyte ghosts. A series of experimental OP's were also synthesized to test the reactivation potential of the oximes, one of which phosphorylate AChE with the same moiety as the nerve agent sarin. The sarin surrogates, 4-nitrophenyl isopropyl methylphosphonate (IC50=22nM), phthalimidyl isopropyl methylphosphonate (IC50=36nM), or phthalimidyl diethyl phosphate (IC50=38nM) were used to inhibit approximately 80% of AChE and degrade quickly in aqueous solution to prevent re-inhibition of reactivated AChE. Tissues were treated with an oxime and reactivation of AChE was measured by a discontinuous cholinesterase assay. Lipophilicity, reflecting the potential for crossing the blood-brain barrier, was determined by n-octanol/water partition coefficients and varied among the oximes with some up to 30 fold greater than 2-PAM. Reactivation varied among the different oximes (39-80%) as well as among the OP's and between the two different tissues. A few of the novel oximes showed combined higher lipophilicity and reactivation potential approaching that of 2-PAM. Supported by DTRA 1.E0056_08_AHB_C.

134 A SULFUR MUSTARD AND THERMAL SUPERFICIAL DERMAL INJURY PIG MODEL.

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Sulfur mustard [bis (2-chloroethyl) sulfide; HD] is an alkylating, blistering agent that can produce incapacitating, deep lesions that are slow healing and tend to become infected. Treatment strategies for burns vary and are based on those for thermal injuries, yet healing is prolonged with contracture and scarring. Eight anesthetized female Yorkshire-cross weanling pigs were used to develop a thermal and HD superficial dermal injury model for comparison of lesion progression and healing between the two etiologies. Two ventral abdominal sites on one side were exposed to 400 microliter undiluted HD for 8 min, and sites on the opposite side treated with a 70 degree C brass rod for 45 s or 60 s. Pigs were given a regimen of buprenorphine to control pain. Lesions were evaluated for size, and given a Modified Draize score (0 = unchanged from normal skin, 1 = mild/moderate edema and/or erythema, 2 = severe edema and/or erythema, 3 = focal areas of necrosis with tissue still pliable, 4 = eschar covering > 75% of the site). At 48 hr following exposure, animals were euthanatized and skin samples excised for histopathology. Thermal injuries tended to be similar in size to those caused by HD. Modified Draize scoring of thermal injuries tended to be more severe (3-4) than those caused by HD (1-2). Edema from thermal injuries was minimal or moderate at 48 hr and nonexistent to minimal for HD injuries. Histopathology of both lesions indicated a superficial dermal injury with 100% basal cell necrosis, ~ 33% dermal coagulation, and a depth of injury ~33% into the dermis. With thermal burns, however, vessels are coagulated with no hemorrhage, inflammation is almost restricted to the subdermis, and inflammation appears to arise from vessels at the adipose-dermal junction.
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135 MECHANISM OF INHIBITION OF THIOREDOXIN REDUCTASE IN LUNG EPITHELIAL CELLS BY THE VESICANT 2-CHLOROETHYL ETHYL SULFIDE.

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Sulfur mustard is a potent vesicant known to damage lung tissue. Toxicity is the result of alkylation of critical proteins that maintain the integrity of the lung, resulting in inflammation and oxidative stress. Thioredoxin reductase (TrxR) is a selenocysteine-containing flavoprotein that catalyzes the NADPH-dependent reduction of oxidized thioredoxin and plays a key role in regulating cellular redox homeostasis. We examined the effect of 2-chloroethyl ethyl sulfide (CEES), a model sulfur mustard vesicant, on thioredoxin reductase in lung epithelial cells to determine if altering its activity contributes to oxidative stress. CEES treatment of human lung A549 epithelial cells resulted in a marked concentration- and time-dependent inhibition of TrxR activity. To analyze mechanisms mediating this effect we used highly purified rat liver TrxR. CEES caused a time-, NADPH-, and concentration-dependent inhibition of TrxR activity. Inhibitory effects of CEES were also observed in NADPH-reduced TrxR, but not oxidized TrxR, suggesting that the vesicant covalently modified the reduced selenocysteine-containing active site of the enzyme. This is supported by our findings that the reaction of TrxR with iodoacetamide, which selectively reacts with free sulfhydryl or seleno groups on proteins, was markedly inhibited by CEES. Moreover, CEES was significantly less effective in inhibiting a recombinant TrxR in which selenocysteine was replaced by cysteine. These data demonstrate that sulfur mustard vesicants can target TrxR and this may be an important mechanism by which these compounds induce oxidative stress.
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136 RADIATION PROTECTION AGAINST EARLY AND LATE EFFECTS OF IONIZING RADIATION BY GENISTEIN.

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The protective effects of the soy isoflavone genistein against early and late radiation injury after total body irradiation was investigated in female C57BL/6J mice. Little attention has been given to protection against late effects that may develop after survival from the acute radiation syndrome, as is the case when a radiation countermeasure agent has been administered. Here we examined the radioprotective activities of genistein against both acute lethality and delayed radiation-induced lung injury. Mice were administered a single subcutaneous injection of vehicle (PEG-400) or genistein (200 mg/kg) 24 h before sham irradiation or 7.75 Gy irradiation (0.6 Gy/min, Cobalt-60). Experimental groups were: No treatment + Sham (NC), Vehicle + Sham (VC), Genistein + Sham (GC), Radiation only (NR), Vehicle + Rad (VR), Genistein + Rad (GR). First generation lung fibroblasts were analyzed for micronuclei 24 h post-irradiation. Histology was used to identify fibrotic plaques. Proteins with altered expression in fibrosis (TGFβRI and II, ACE, and COX-2) were evaluated at 90, 120, and 180 days post-irradiation by Western blot. Thirty-day survival rates for NR, VR, and GR were 23%, 53%, and 92%, respectively. GR mice had significantly reduced micronuclei compared with NR or VR. At 90 days post-irradiation small fibrotic plaques were present in lungs of NR but not GR. GR and NR had reduced TGFβRII and COX-2 at 90 days; genistein prevented NR-induced reduction of TGFβRI. By 180 days post-irradiation all proteins had normalized. Genistein-treated mice that survived radiation-induced hematopoietic injury exhibited reduced lung damage compared to NR. These data demonstrate that genistein prevented early mortality and delayed radiation-induced lung damage in C57BL/6J mice.

137 ACUTE TOXIC EFFECTS OF VX VAPOR IN RATS – INTERACTIONS OF EXPOSURE CONCENTRATION AND DURATION.

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The nerve agent VX is the most toxic acetylcholinesterase inhibitor known to man. While there are isolated reports on the inhalational toxicity of VX in animals and human, there is low to moderate degree of confidence on the current proposed LCt50 values for animals (67 mg.min/m3; 12 min exposure) and human (7 mg.min/m3; 10 min exposure). Key concerns raised over cited exposure studies include the absence of reliable VX challenge concentrations, failure to address toxic effects of carrier solvent (i.e., benzene) and paucity of information on interactions between exposure concentration and duration. To resolve current uncertainty on VX acute toxicity over different durations of exposure, we performed a series of nose-only acute inhalation studies on adult male and female Sprague-Dawley rats. Challenge studies were carried out for 4 different exposure durations, with 6 VX concentrations evaluated per exposure duration and 10 rats tested per concentration. VX vapor, conditioned to 30OC and 50% RH, is channeled into the nose-only inhalation chamber and measured on-line with a Minicam GC-PFPD analyzer. Mortality of the exposed rats was followed for 14 days post exposure to derive AEGL 3 values. For AEGL 2 data, on-line plethysmography was used to derive changes in tidal mid-expiratory flow (EF50) and tidal volume. For AEGL 1 analysis, animals were evaluated for 50% decrease in pupil size immediately post VX-challenge.
Unlike sarin, male rats were determined to be more sensitive to VX vapor toxicity while the solvent vehicle did not result in any mortality over 14 days. From 14-days mortality studies on male rats, for 10 min exposure duration, we determined that current AEGL 3 values are over-conservative by 3-fold. However, for exposure durations of 60 minutes and beyond, current recommendations under predict toxicity of inhaled VX up to 2-folds. Data for VX AEGL 2 were found to under-predict slightly (1.2 fold) the respiratory depressive effects of inhaled VX. Similar findings were made for current AEGL 1 values.

138 QUANTITATION OF THE BIOMARKER ETHYL METHYLPHOSPHONOFLUORIDATE IN RED BLOOD CELLS FOLLOWING AN ACCIDENTAL EXPOSURE TO THE NERVE AGENT VX.

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A rapid and sensitive method for determining exposure to the chemical warfare nerve agent VX is described in which the biomarker ethyl methylphosphonofluoridate (VX-G) is measured in red blood cells (RBCs) following treatment with fluoride ion using isotope-dilution gas chromatography tandem mass spectrometry (GC-MS/MS). The aforementioned analyte and a deuterated analog were isolated via solid phase extraction and detected using ammonia chemical ionization in the multiple reaction monitoring mode. A good linear relationship was obtained in the quantitative concentration range of 4 ng/mL to 1000 ng/mL with an absolute detection limit of <1 picogram of VX-G on column. The method has been applied to the analysis of RBCs from a laboratory worker accidentally exposed to VX vapor

taken at 1 day, 6 days, 8 days, 20 and 27 days post exposure. Detection and quantitation of VX-G was possible in samples taken as late as 27 days following exposure demonstrating the utility of the method for retrospective recognition. These results are consistent with earlier studies on IV administration of VX in human volunteers where red blood cell cholinesterase depression returned to only 80% to 90% of normal within 14 days, while plasma cholinesterase depression returned to 100% of normal within one week.

139 EFFICACY COMPARISON OF RSDL, M291 SDK, 0.5% BLEACH AND 1% SOAPY WATER CHALLENGED WITH SOMAN, CYCLOSARIN, VX, AND RUSSIAN VX.

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This study compares the efficacy of four decontamination products challenged by nerve agents. Guinea pigs were anesthetized and challenged with agent. Two minutes after challenging, the exposed sites were either decontaminated by one of the four decontaminating materials or left un-treated and the 24-hour survival recorded. The protective ratio (PR) was calculated from the derived median lethal dose-response curves established for each agent and non-decontaminated control animals. RSDL provided superior efficacy against soman (GD), cyclosarin (GF), VX, and Russian VX (VR) compared to the other decontamination products tested. The 1% soapy water and 0.5% bleach, though not as protective as RSDL, provided good and equivalent protection against GD, GF, and VX. Against VR exposure, the 0.5% bleach was twice as effective as soapy water. The M291 SDK was the least effective decontaminant overall, providing modest protection against GD and GF and hardly any protection against VX and VR. Additional experiments were conducted with VX where decontamination was delayed up to 60 minutes. In these experiments, guinea pigs were challenged with a constant 0.625 mg/kg (5 LD50) dose of VX. Decontamination was delayed for times from 2 to 60 minutes. The results from these experiments using RSDL with 63 animals showed that there were no observed deaths through a 25-minute delay. The data was analyzed using probit analysis by substituting the delay time for the mg/kg challenge dose. The delay time associated with 50 % lethality was 30.6 (95% C.I. 29.6 – 31.6) minutes with a probit slope of 58. Preliminary data with just a limited number of animals suggests that 0.5% bleach and 1% soapy water will have about the same delayed decontamination results as RSDL. The M291 SDK was not included in these experiments because of its poor performance.

140 DEVELOPMENT OF SELECTIVE NANOPOROUS SORBENTS FOR RADIONUCLIDE DEPORPORATION.

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Novel decorporation agents are being developed to protect against radiological terrorist attacks (i.e. dirty bombs). Nano-engineered solid sorbents for radionuclide chelation therapy were developed and validated *in vitro.* These sorbents, known as the self-assembled monolayer on mesoporous supports (SAMMS), are hybrid materials where differing organic moieties are grafted onto mesopourous silica (SiO_2). Batch contact experiments focused on the evaluation, and optimization of SAMMS for capturing single and multiple radionuclides (Pu, U, Am, Co and Cs). In these experiments a number of parameters were evaluated including: sorption affinity (Kd), kinetics, selectivity and stability. An isomer of hydroxypyridinones on mesoporous silica (3,4-HOPO-SAMMS) demonstrated the highest *in vitro* Kd values in human blood and plasma for decorporation of U, Am, and Pu and outperformed the FDA-approved actinide ligand diethylenetriaminepentaacetate (DTPA) by a factor of 1000-fold. Batch decorporation was extremely fast (complete ~10 min), with optimal performance at pH 6-8, no evidence of protein fouling and less than 0.2% leaching of the Si from the SAMMS. The 3,4-HOPO-SAMMS (in bead format) was further evaluated to determine a capacity for extracorporeal chelation of radionuclides by externally circulating the plasma through SAMMS material contained in a microfiltration device. Plasma (~50 mL) was spiked with known concentrations of radionuclide (U, Am, or Pu), gently stirred and pumped (2 ml/min) from a mixing reservoir into the device. The reservoir was sampled over time to quantify the amount of radionuclide present. Decorporation of U, Am, and Pu from plasma was rapid and complete with $t_{1/2}$ of 18, 24 and 143 min, respectively. 3,4-HOPO on silica beads outperformed the best commercially available actinide sorbent, Diphonix resins. This study suggests that SAMMS materials are ideal extracorporeal chelators. Future studies are focused on increasing decorporation efficacy and *in vivo* evaluation in animals. (Supported by NIH/NIAID grant R01 AI0 74064-01)

141 SUB-ACUTE SARIN EXPOSURE TRIGGERS NEUROCHEMICAL AND NEUROPATHOLOGICAL CHANGES IN THE RAT BRAIN.

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We previously reported that an acute exposure to an LD50 dose of sarin induces early neuropathological changes in the adult rat brain. In this study, we investigated the delayed effects after seven days of a single intramuscular injection of the sarin at doses of 1, 0.5, 0.1 and 0.01 X LD50 (100 mg/kg) on the adult rat brain. Both sarin-treated and vehicle-treated (control) animals were for: (i) plasma butyrylcholinesterase (BChE) activity; (ii) brain acetylcholinesterase (AChE) activity, (iii) m2 muscarinic acetylcholine receptor (m2 mAChR) ligand binding; (iv) histopathological changes in the brain using H&E staining, and microtubule-associated protein (MAP-2), glial fibrillary acidic protein (GFAP), 3-nitrotyrosine(3-NT) immunostaining, and TUNEL and ssDNA assays. Animals treated with 1 x and 0.5 X LD50 sarin exhibited a significant decrease in forebrain and brainstem AChE activity and m2 mAChR ligand binding. In addition, animals treated with 0.5 X LD50 displayed reductions in the density of surviving neurons with reduced MAP-2 immunoreactivity and increased GFAP immunopositive element expression in the motor cortex, the hippocampus and the cerebellum. Further, scattered degenerating neurons were observed in animals treated with 0.5 X LD50, particularly in the motor cortex and the hippocampus; this was characterized by an increased 3-NT immunoreactivity; the presence of cells positive for TdT-mediated dUTP nick-end labeling (TUNEL), and an single stranded ssDNA. In contrast, neither the 0.1 nor the 0.01 X LD50 treatments induced neurodegenerative changes in the above brain regions at this time point. Collectively, the above results suggest that apoptosis plays a role in the sarin-induced delayed apoptotic neuronal degeneration in many regions of the brain, which is dose-dependent. Supported by the USAMRMC DAMD 17-98-1-8027.

142 EFFECTIVENESS OF RSDL TOPICAL SKIN DECONTAMINANT AT REDUCING MORTALITY IN RATS WHOLE-BODY EXPOSED TO VX VAPOR.

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O-Ethyl S- [2-(diisopropylamino) ethyl] methylphosphonothiolate (VX) is a low volatility organophosphorous compound (OP) that persists on exposed surfaces and has a substantial percutaneous component to its toxicity. The primary objective of this study was to determine the effectiveness of the topical decontaminant RSDL (Reactive Skin Decontaminant Lotion) in 3 different post-exposure scenarios. Male and female rats were whole-body exposed to lethal dosages of VX vapor for 10 min. Following exposure, topical RSDL was applied to the skin and fur at either 20 or 50 min post-exposure. A third group of rats were exposed but not decontaminated and used for comparison. Comparisons were made of the calculated 24 hour LCT_{50}'s (median lethal dosages) for the three groups. The effectiveness of RSDL at reducing mortality varied with respect to the time of RSDL application following exposure. When RSDL was applied at 20 or 50 min post-exposure, it significantly shifted the LCT_{50}'s upward compared to the LCT_{50} of the untreated group. There were no significant differences in acetylcholinesterase (AChE) activity attributable to gender or between either of the decontamination groups at any of the dosages tested. Residual AChE activity at all dosages tested was 15% or less of non-exposed controls. The results of the present study suggest RSDL is an extremely effective topical decontaminant of VX if applied within 60 min of VX exposure.

143 RICIN STABILITY AND BIO-ACTIVITY AFTER TREATMENT AS DETERMINED BY A NEUTRAL RED UPTAKE ASSAY.

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Ricin has remained a significant potential biological threat agent due in part to its wide availability and ease of extraction from the castor bean. It is a glycoprotein made up of A- and B- chains with the B-chain facilitating the transport of the lectin into the cell. Once inside, it kills the cell by inhibiting protein synthesis. Ricin is considered extremely toxic to man by multiple routes of exposure and although it is

considered quite stable, it can be denatured and inactivated by factors such as temperature and pH. It is essential to have techniques developed that not only can detect and identify the protein toxin, but to determine its activity. We previously reported our adaptation of the well-known Neutral Red Uptake (NRU) assay using BALB/c 3T3 murine fibroblasts for the measurement of ricin's toxicity after being subjected to various treatments (Madren-Whalley et al., 2007 SOT poster). We have applied this protocol to ricin (Vector Laboratories, product L-1090) incubated at pH 7 in 10 mM phosphate buffer at 25 degrees Celsius for 0, 4, 18, and 32 days and found that ricin's cytotoxicity remained unchanged over this time frame. This sensitive cell-based assay is a valuable tool to evaluate decontamination procedures and to determine the toxicity of unknown samples. Support: Defense Threat Reduction Agency Project BA05TAS080 & National Research Council/DTRA post doctoral program.

144 STUDIES ON THE EFFECT AND MECHANISM OF LONG-TERM EXPOSURE TO DEPLETED URANIUM ON BONE METABOLISM AND BONE MINERAL INJURY.

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Objective Depleted uranium (DU) is a kind of radioactive heavy metal which can enter into the body by inhaling (aerosols), drinking, eating and wounds and cause chemical and/or radioactive toxicity. In this study, the biological characters and mechanism of DU damage to bone were analyzed, to afford medical therapy for DU or other radioactive heavy metals'combined damage to bone in experimental side. Methods Sprague Dawley rats were surgically implanted in gastrocnemius muscle with DU pellets at three dosage levels. At 90d after DU implantation, the bone mineral density and bone biomechanics were analyzed, and the pathological changes were observed by microscope and measured by bone histomorphomety. Meanwhile, renal dysfunction, the 1α-hydroxylase activity in kidney, and the 1α,25(OH)2D3 level in blood were analyzed to discuss the mechanism of bone lesion. Results At the 90th day after implantation, the BMD in DU implanted animals were significantly lower than those of control group, and with a high correlation to the implanted DU doses. In addition, the lumbar compressive loads and femoral three-point bending test in DU implanted rats were significantly declined compared with the control group, and alterations of the trabecular bone structure were observed in DU implanted rats. Bone metabolism was disturbed in the rats implanted with DU, Ca and ALP level in blood decreased, and level of urinary deoxypyridinoline(Pyd)/Cr increased. Otherwise, chronic DU exposure could induce obvious renal damages as the dose of DU increased. And then, the activity of renal 1α-hydroxylase declined, vitamin D synthesis was not enough and 1α,25(OH)2D3 level in blood declined. Conclusions DU can cause bone damage such as the disturbance of bone metabolism, destruction of bone microarchitecture, loss in bone mass and biomechanical properties. Decline of 1α-hydroxylase activity and deficiency of vitamin D may be the mechanism of bone metabolic disorder caused by renal injury after DU exposure.

145 MACHINE LEARNING ALGORITHMS IDENTIFY EXPOSURE AND POTENTIAL MECHANISMS OF GF VAPOR TOXICITY IN RAT BRAIN.

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Following potential exposure of more than 100,000 troops to a sarin/cyclosarin (GB/GF) mixture during the first Gulf War, there has been increasing concern regarding the potential for persistent effects of low-level exposure to these agents. Recently, it has been demonstrated that exposures at levels which do not result in overt clinical signs, may produce subtle neurocognitive deficits, as well as significant long term neurophysiological changes. The aim of this study was to determine whether several machine learning methods could determine which blinded gene expression samples were from brains of rats exposed one week prior to low levels of the aerosolized nerve agent GF. Specifically, neural networks, naïve Bayesian classifiers, and simple class signatures were applied to microarray-derived transcriptomic data from the brains of male and female Sprague-Dawley rats exposed by whole body inhalation to peri- and sub-miotic doses of the nerve agent GF. This manuscript describes the relative utility of these methods with respect to classifying the GF exposure in rats. Additionally, analysis of the most informative genes has identified several potential markers of exposure which could be used for further study of molecular mechanisms of GF toxicity in the brain.

146 UPREGULATION OF THE 5-LIPOXYGENASE LEUKOTRIENE BIOSYNTHETIC PATHWAY BY THE VESICANT 2-CHLOROETHYL ETHYL SULFIDE IN MOUSE KERATINOCYTES.

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Dermal exposure to sulfur mustard chemical warfare agents induces an inflammatory response followed by edema and blister formation, resulting in persistent tissue damage. Previous work from our group has shown that in the mouse ear vesicant model, inflammation induced by the half mustard 2-chloroethyl ethyl sulfide (CEES) is associated with a marked increase in expression of 5-lipoxygenase (5-LOX) and production of leukotriene B4 (LTB4). Leukotrienes are potent mediators of inflammation and are also known to regulate epidermal cell growth and differentiation. Using a mouse keratinocyte cell line (PAM212), we generated an in vitro epidermal model to analyze the effects of CEES on the leukotriene biosynthetic pathway. Keratinocytes were grown on a semi-permeable membrane with the basolateral side in contact with medium and the apical side exposed to air, creating an air-surface interface. CEES (100-1000 μM) was applied to the apical surface to simulate actual dermal exposures. CEES upregulated 5-LOX mRNA (3-fold) and protein expression in a dose-dependent manner as determined by real time PCR and Western blotting, respectively. Leukotriene A4 hydrolase and leukotriene C4 synthase mRNA expression were also significantly increased (up to 12-fold) as were keratinocyte receptors for LTB4 and LTC4 (3-5 fold). These data show that CEES is a potent regulator of the 5-LOX biosynthetic pathway. Upregulation of these enzymes and production of leukotrienes are likely to play an important role in the mechanisms of skin toxicity following exposure to sulfur mustards. Supported by ES005022, ES004738 and the NIH CounterACT Program through NIAMS U54AR055073. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the federal government.

147 THIOREDOXIN REDUCTASE REDUCES SULFUR MUSTARD IN VITRO TO YIELD CARBON-BASED FREE RADICALS.

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Recent *in vitro* work from our laboratory proves that sulfur and nitrogen mustards are activated to carbon-based free radicals when their characteristic cyclic sulfonium or ammonium ions undergo enzymatic reduction. Using electron paramagnetic resonance (EPR), we have shown that this reaction can be catalyzed by several pyridine nucleotide-driven flavoenzymes including cytochrome P450 reductase (CYPR) and the reductase domain of neuronal nitric oxide synthase (nNOSR) in the presence of a spin trap. Thioredoxin reductase (TrxR, thioredoxin-disulfide reductase [EC 1.8.1.9]) is a cytosolic flavoenzyme that catalyzes the NADPH-dependent reduction of the active site disulfide in oxidized thioredoxin. Recent work [Gray, J.P., et al. J. Biol. Chem. 282 (2007) 7939-7949] has identified TrxR as the enzyme that cyclically reduces the herbicide paraquat to the bipyridyl radical which redox cycles with O2, forms oxygen radicals and leads to lung damage. Radical generation with paraquat also occurs under the influence of CYPR and the nNOSR domain. However, it appears the TrxR is a more effective catalyst of the reaction. The obvious parallel caused us to test mustard "onium" ions with TrxR. We prepared a 10mm flat cell containing 4.0 mM sulfur mustard, 2.4 mM NADPH, 0.034 μM TrxR from rat liver, 0.1 M KH2PO4 buffer, pH 7.5 made with Chelex treated deionized water and the spin trap 4-POBN (α-[4-pyridyl-1-oxide]-N-tert-butylnitrone) at 1.03 M. We use a Bruker EMX EPR spectrometer equipped with a 9118 sample cavity. Instrument settings: microwave power 20.00 mW; modulation amplitude,1.00 G; center field, 3480.00 G; time constant, 1.250 ms; 4 scans @ 204.8 s/scan; receiver gain, 1.26 x 104. The outcome was a triplet of doublets with hyperfine splitting constants of aH = 2.665 and aN = 15.425 determined by simulation. These values compare favorably with results obtained by EPR using other mustards and other reductases. These compounds share a common path of toxicant activation, so inhibitors of TrxR may be therapeutic for HD toxicity.

148 EFFECTS OF VX ON ACOUSTIC STARTLE RESPONSE AND OPERANT BEHAVIOR IN RATS.

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The present study evaluated the dose-response effects of subacute exposure to sublethal doses of nerve agent VX on the acoustic startle response (ASR) and operant behavior of rats. ASR baseline was established and rats were exposed to 2 consecutive daily doses of fractions (0.2, 0.4, and 0.6) of the established LD50 of VX. ASR

testing continued for 2 days post-exposure. Subsequently animals were dietary restricted and trained to respond for food under an autoshaping procedure. Following the establishment of lever pressing, a series of escalating fixed ratio (FR) schedules was introduced. Thereafter, four sessions were conducted under differential reinforcement of low response rate 10-sec schedule. Finally, an ascending and descending series of geometric progressive ratio schedules were evaluated. VX decreased the magnitude of the startle response to 100 dB pulses on injection days for all VX groups and decreased the startle response to 120 dB pulses on injection days for the 0.4 and 0.6 LD50 groups. In addition, VX increased the latency to peak startle magnitude in the animals receiving 0.4 and 0.6 LD50. There were no significant dose-related effects on prepulse inhibition. There were no significant dose-related differences on the acquisition of lever pressing via autoshaping or responding under DRL 10-sec schedules of reinforcement. There were significant dose-related effects on reinforcement rate during the FR schedules and on break point under the geometric progressive ratio schedules. Taken together, these data indicate that there are few persistent effects of subacute VX exposure on the acquisition of operant behavior. This research was supported by the Defense Threat Reduction Agency – Joint Science and Technology Office, Medical S&T Division.

149 NITRITE-MEDIATED ANTAGONISM OF CYANIDE INHIBITION OF CYTOCHROME C OXIDASE.

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Cyanide produces a rapid inhibition of cytochrome c oxidase (CcOX), thereby producing an inhibition of mitochondrial oxidative phosphorylation. Amyl nitrite and sodium nitrite antagonize cyanide toxicity in part by oxidizing hemoglobin to methemoglobin, which then scavenges cyanide. Methemoglobin binds cyanide with a greater affinity than CcOX. Methemoglobin generation is thought to be the primary mechanism by which nitrite antagonizes cyanide. Under biological conditions, nitrite can also release nitric oxide (NO) and we have shown that NO directly antagonizes cyanide inhibition of CcOX. In this study, nitrite-mediated antagonism of cyanide was examined in N27 rat mesencepalic cells. Sodium nitrite ($NaNO_2$) increased cellular NO levels in a concentration-dependent manner and the NO scavenger 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (PTIO) reversed the nitrite-mediated increase in NO. Cyanide inhibition of cellular oxygen consumption and CcOX activity were antagonized by 100 μM $NaNO_2$. KCN (20 μM) inhibited cellular respiration by 57% (IC_{50} = 13.2 ± 1.8 μM). Nitrite (100 μM), on the other hand, produced 76% inhibition of cellular respiration, which was partially reversed by PTIO (200 μM). When KCN (20 μM) and $NaNO_2$ (100 μM) were combined, cellular oxygen consumption increased to control (0.06 ± 0.02 VO_2/ min for KCN + $NaNO_2$ vs. 0.07 ± 0.01 VO_2/ min for control). PTIO (200 μM) reversed the nitrite-mediated antagonism of cyanide during oxygen consumption. CcOX enzymatic assays further confirmed that nitrite antagonizes cyanide (CcOX rates were 0.18 ± 0.08 μmol/ min for KCN and 0.46 ± 0.16 μmol/ min for KCN + $NaNO_2$). It is concluded that in addition to methemoglobin formation, $NaNO_2$ antagonizes cyanide inhibition of CcOX through generation of NO, which directly alters KCN-mediated inhibition of CcOX. (Supported in part by NIH Grant ES04140.)

150 TREATMENT WITH TERTIARY OXIMES PREVENTS SEIZURES AND IMPROVES SURVIVAL FOLLOWING SARIN INTOXICATION.

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Organophosphorus nerve agents, such as sarin, are potent cholinesterase (ChE) inhibitors. While current pretreatment (pyridostigmine) and therapies (atropine sulfate and 2-PAM) for nerve agent intoxications can reduce lethality, these treatments do not prevent convulsions and neuropathology. These persistent toxicities could be due to the quaternary structure of 2-PAM, which is unable to reactivate nerve agent-inhibited ChE in the CNS. We have examined the capability of the tertiary oximes monoisonitrosoacetone (MINA) and diacetylmonoxime (DAM), in comparison with several quaternary oximes (2-PAM, HLo7 and MMB-4), to prevent or eliminate sarin-induced electroencephalographic (EEG) seizure in a guinea pig model. Animals were pretreated IM with pyridostigmine bromide 30 min prior to challenge SC with 2.0 x LD50 of sarin, and treated IM one min later with a combination of atropine sulfate (2.0 mg/kg) and a single dose of quaternary oxime or a varied dose of MINA or DAM. With the quaternary oximes (2-PAM, MMB-4 or HLo7), all animals (100%) developed continuous seizure activity and 20 - 50% of animals survived 24 hr. With MINA at doses of 20, 26, 35, 46 and 60 mg/kg, 0, 9, 17, 60, and 75% of animals, respectively, never exhibited EEG seizure activity and 43, 64, 75, 90, and 100% of these animals, respectively, survived 24 hr. Similarly, with DAM at doses of 41, 73, 129 and 231 mg/kg, 0, 17, 67 and 100%, respectively, of animals never exhibited EEG seizure activity and 71, 83, 100 and 100% of these animals, respectively, survived 24 hr. The anticonvulsant ED50 (with 50% confidence limits) for MINA in the presence of atropine sulfate (2.0 mg/kg) was 36.65 (0.00 – 76.30) mg/kg, whereas the anticonvulsant ED50 for DAM was112.51 (83.78 – 178.87) mg/kg, showing that MINA was significantly more potent than DAM under similar conditions. These data show that the tertiary oximes improved survival and eliminated seizures following sarin intoxication.

151 SULFUR MUSTARD ANALOG-CAUSED ACTIVATION OF SIGNALING CASCADES IN SKH-1 HAIRLESS MOUSE SKIN.

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Sulfur mustard (HD) is a potent vesicant first used as a chemical weapon in World War I and more recently during the Iran-Iraq conflict, and therefore, remains a significant military and civilian threat. Apart from causing ocular and respiratory organ damage, skin is the primary and direct target organ for sulfur mustard. Despite extensive research effort, there is still no simple and reproducible in vivo model to evaluate sulfur mustard-induced skin injury and associated molecular targets. Here, using female SKH-1 hairless mice, we studied the involvement of extracellular signal regulated kinase 1/2, p38 and JNK and nuclear factor-κB and AP1 signaling in topically applied 2-chloroethyl ethyl sulfide (CEES)-caused skin toxicity. The activation of various signaling molecules was assessed using immunohistochemistry, western blot and electrophoretic mobility shift assays. Our results showed a dose-response effect of CEES (at 1.0, 1.5 and 2.0mg/mouse doses for 12h) on the activation of MAP kinases and transcription factors NF-κB and AP1. The effective 2mg CEES dose was next employed in a time-response (3, 6, 9, 12, 24, 48, 72 and 168h after CEES treatment) study, which showed similar activation of MAP kinases and both NF-κB and AP1. The activation and expression pattern of these molecules showed a close correlation with the level of CEES-induced skin injury. Collectively, our results indicate the involvement of MAP kinase pathways, and both NF-κB and AP1 activation in CEES-induced skin injury in female SKH-1 hairless mice. Together, these findings identify new molecular targets involved in CEES-induced skin toxicity in an in vivo model providing valuable markers for activation of inflammation; potentially useful to screen compounds to treat/prevent sulfur mustard toxicity.

152 IMAGING EARLY PATHWAYS OF SULFUR MUSTARD TOXICITY.

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Topical exposure to sulfur mustard (HD, bis-2-chloroethyl sulfide) produces blisters at the epidermal-dermal junction of skin after a dose-dependent latent phase of 8-24 h. To develop effective postexposure treatments against this agent requires a fundamental knowledge of the early damage that precedes and initiates blister formation. In pursuit of that knowledge, we exposed human epidermal keratinocytes (HEK) to 400 μM HD for 5 min. Using image-based search strategies, we applied multiphoton microscopy, immunofluorescent probes, and gene-array analysis to search for the earliest lesions of HD-induced toxicity. Results from 3 postexposure profiles showed that HD caused reproducible, statistically significant changes in the expression of specific genes that are related in space and time to pathways signaling the onset and dissolution of an abnormal F-actin cytoskeleton. From that data, we explored the possibility that the earliest lesions potentiate vesication by disrupting cytoskeletal integrity, focal adhesions and the interactive adhesion complex of epidermal basal cells. Within 15 min of exposure, HEK showed proteolytic detachment (HD-avoidance) responses, e.g., up-regulation of TMPRSS4, a transmembrane protease with extracellular trypsin-like activity, and up-regulation of MKNK2, a MAP kinase-interactive ser/thr kinase-2, which modifies cell shape and cytoskeletal maturation. By 1 h postexposure, the progressive weakening of HEK attachments included loss of stress fibers and down-regulation of α6β4 integrin receptors, laminin-5's α3 chain and β-catenin. Analyses indicate that early progressive disruption of F-actin, focal adhesions and associated signaling pathways are major components of HD-induced pathology and blistering. These studies are providing an essential understanding of early HD targets and their effects on the window of opportunity for successful intervention and treatment.

153 PROTEOMIC ANALYSIS OF TYROSINE-PHOSPHORYLATED PROTEINS FROM RAT HIPPOCAMPUS FOLLOWING SOMAN EXPOSURE.

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Current therapeutics used to treat the effects of organophosphorous (OP) nerve agent exposure are inadequate to suppress OP-induced neuropathology. To identify targets for the development of therapeutics, it is important to identify alterations in

the protein profile of OP-exposed brain tissue. Alterations in tyrosine phosphorylation represent signaling cascades responsive to OPs. Alterations in total protein abundance can provide insight into biological processes responsive to OPs. We used proteomic techniques to evaluate changes in tyrosine phosphorylation and total protein abundance following exposure to the OP nerve agent soman (GD). For detection and analysis of proteins phosphorylated on tyrosine residues, male Sprague-Dawley rats were pretreated with the oxime HI-6 (125 mg/kg, sc) and challenged with GD (120 ug/kg, sc) or with saline. One minute after challenge, animals were treated with atropine (2.0 mg/kg, im) and euthanized 30 or 120 minutes after exposure. Hippocampi were homogenized and analyzed by Western blotting with anti-phosphotyrosine antibodies or immunoprecipitated with antiphosphotyrosine antibodies and separated by 2D electrophoresis. Changes in total protein abundance were evaluated using 2D fluorescence difference gel electrophoresis (2D DIGE). For 2D DIGE, rats were exposed to GD (30 ug/kg or 75 ug/kg, sc) or to saline. Hippocampi were harvested at 30 min, 1, 2 and 4 hr post-exposure and homogenized, and total protein was fluorescently labeled prior to 2D electrophoresis and image analysis. MALDI-TOF-TOF mass spectrometry was used to identify specific proteins detected by these approaches as changed due to GD exposure. Two groups of proteins were identified. One group represents proteins known to function in vesicle transport; the other represents proteins known to function in maintenance of the cytoskeleton. This work demonstrates that proteomic approaches can identify molecular responses following GD exposure.

154 PHYSIOLOGICAL CHANGES IN C3H/HEJ MICE AFTER MICROINSTILLATION INHALATION EXPOSURE TO STAPHYLOCOCCAL ENTEROTOXIN B.

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Staphylococcal Enterotoxin B (SEB) is a potential biological warfare agent that has the potential to cause mass casualties in war or by terrorist activities. Although inhalation is the most likely route of exposure of SEB when used as a biological weapon, there are few studies assessing inhalation toxicity. We developed a microinstillation model of SEB inhaltion exposure in C3H/HeJ mice. A custom made microcatheter was inserted into an endotracheal tube, positioned 0.5 cm above the tracheal bifurcation and aerosolized 0.2λ of SEB per pulse with a total of 40 pulses over 1 min. Four groups of mice were challenged with; saline, 0.3LD50, 0.5LD50 or 0.7LD50 [1LD50=1.6ug/g] SEB and recovered for 24 hours. All mice survived SEB exposure and were monitored for behavioral changes; a necropsy was later performed to evaluate toxicity. All treated groups lost around 5.0±3.0% of their body weight. Visual lung injury was observed after necropsies with an increase in bronchoalveolar lavage fluid cells. A total of 35.4x10e4±12.3x10e4 cells/ml was observed in the control group with 1.42x10e4±3.57x10e4 dead cells/ml. These numbers showed a dose dependant increase to 59.1x10e4±74.1x10e4 total cells/ml and 11.0x10e4±16.5x10e4 dead cells/ml in the 0.5LD50 group. Blood collected via cardiac puncture showed dose dependant variation in immune cell numbers. The numbers of neutrophils increased in a dose dependant fashion, with the control group having an average of 0.91x10e3±0.45x10e3 cell/ul and increased to 1.8x10e3±0.53x10e3 cells/ul in the 0.7LD50 group. Lymphocytes numbers decreased in a dose dependant manner. This data indicates that C3H/HeJ mice are susceptible to SEB intoxication by inhalation of SEB and causes various pathological and physiological changes.

155 SULFUR MUSTARD INDUCED CHANGES IN LAMININ-332 IN THE MOUSE EAR VESICANT MODEL (MEVM).

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The heterotrimer laminin-332 (formerly LN-5) is a skin protein important to the structural integrity of skin. It is composed of three polypeptide chains named α3, β3, and γ2. In vitro evidence suggests that human acute wound fluid upregulates all three gene products so we decided to explore this phenomenon in vivo. Mouse ear skin was wounded by topical exposure to the chemical agent, bis(2-chloroethyl) sulfide (sulfur mustard, SM) and a time-course study followed to examine the laminin-332 chains as potential biomarkers of injury. Mouse skin biopsies 6, 12, 24, 72, and 168h after SM exposure were visualized by H&E staining and demonstrated worsening skin damage with increasing time. Immunohistochemistry of tissue sections with chain-specific antibodies showed overlap of the three chains in the vicinity of the laminin-332 producing basal keratinocytes. Laminin-332 also appeared to line the sebaceous glands. Real-time PCR indicated an initial drop in mRNA levels early (within 24 hours), followed by increased levels for all three chains at 72h and 168h. The laminin-γ2 chain mRNA levels were about 10 fold higher than the other two chains in the 72 and 168h samples. Western blot analysis of the laminin-γ2 chain indicated a quantitative increase in the laminin γ2 protein over time as well as increased processing of the chain to smaller forms. We believe that the three chains are made de novo to anchor the keratinocytes to the dermis in the normal repair process. We furthermore speculate that processing of laminin-γ2 in the SM-treated ears functions to enhance migration of the epithelial cells.
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156 DEVELOPMENT OF HUMAN *IN VITRO* 3D LUNG MODELS TO ASSESS LUNG INJURY AND EVALUATE THERAPEUTICS AGAINST SOMAN AND SOMAN SIMULANT EXPOSURE.

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Previous toxicology studies, behavioral assessments, and therapeutic evaluations against Chemical Warfare Nerve Agents (CWNAs) have been conducted on animals. Phylogenetically different animals and humans respond differently to CWNA exposure due to variations in the levels of target enzyme activities, detoxification enzymes, cellular metabolism, receptor-mediated electrophysiological effects, and second-messenger signaling. This introduces uncertainties in human risk assessment and difficulties in extrapolating the animal data to humans. We developed an in vitro human 3D physiological alveoli model by air/liquid culture of A549 pulmonary type II pneumocytes on a cell culture membrane insert plate. After the cells were allowed to grow to confluence, the medium in the insert was aspirated to directly expose the cells to air. The model was exposed to increasing doses (25, 50, 75, 100 μL) and (2, 4, 6, 8%) of soman and soman simulant respectively, and evaluated for pulmonary toxicity. Microscopy data showed that cells of nerve agent soman surrogate exposed human lung models were enlarged and elongated compared to the control cells. There was increased cell death in soman surrogate exposed human lung models as well as soman exposed cells. A MTT cytotoxicity assay measuring metabolic activity showed a significant increase in cytotoxicity as the dosage of soman and soman surrogate increased. Exposure to both agents increased permeability of the membrane indicating injury to the barrier. In soman simulant, pretreatment with dexamethasone showed protection in lung cells. These studies show that exposure to organophosphate nerve agents induces pulmonary injury and this will help to cross-species responses to CWNAs.

157 POST-EXPOSURE PROPHYLAXIS WITH A COMBINATION VACCINE-ANTIBIOTIC REGIMEN INCREASES SURVIVAL OF RABBITS CHALLENGED WITH AEROSOLIZED B. ANTHRACIS SPORES.

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Anthrax is considered a biological terrorist and military threat due to the lethal effects by the inhalation route and the stability of B. anthracis spores. The intent of this study was to determine if the use of an accelerated regimen of protective antigen (PA) based vaccine combined with a short antibiotic regimen could provide protection to rabbits challenged with aerosolized B anthracis spores, in support of potential Emergency Use Authorization. Two recombinant PA (rPA) vaccines were tested, as well as Anthrax Vaccine Adsorbed® (AVA, Biothrax), in 3 separate studies. For each vaccine, 4 groups of 18 rabbits were challenged with a targeted 200 LD50s of aerosolized B. anthracis spores and treated for 7 days with levofloxacin (50 mg/kg) and either no or a low, mid or high dose of rPA (10, 30 or 100 ug) or AVA (undiluted, 1:4 and 1:16 dilutions) vaccine on day 0 and 7 post-challenge. A fifth group of 10 rabbits received no treatment. Combined survival of rabbits in groups receiving a combination of levofloxacin and one of the three vaccines was 96% (52/54) at the high vaccine dose, 93% (50/54) at the mid dose, 85% (46/54) at the low dose and 46% (25/54) with no vaccine. Groups receiving antibiotic and vaccine had significantly lower mortality than the antibiotic only group. All non-treated control rabbits died of anthrax. Treatment of two additional groups of unchallenged animals with either antibiotic or vehicle and vaccination (30 μg rPA, Days 0 and 7) indicated that the levofloxacin regimen had no effect on the anti-PA IgG response to rPA vaccine. The results show that accelerated post-exposure vaccination provides additional protection from a lethal B. anthracis inhalation challenge compared to 7 days of antibiotic treatment alone. Levels of serum anti-PA IgG indicated that vaccinated animals rapidly developed protective immune responses in the presence of levofloxacin. These studies demonstrate the development of a standardized animal model to evaluate post-exposure prophylaxis for protection against anthrax.

158 RAPID ASSESSMENT OF THE EFFECTS OF RADIATION EXPOSURE *VIA* FLOW CYTOMETRIC ANALYSIS OF WHOLE BLOOD.

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The human health concerns associated with radiological incidents, whether accidental or intentional, are well documented. However our ability to respond on a clinical level is limited by the lack of technologies that can rapidly and reliably assess the level of radiation that a victim has received. Building on the established response of lymphocyte frequencies to irradiation (IR), the development of a Hematotoxicity Response Matrix (HARM) provides data on the absolute number and an indicator of apoptosis in peripheral blood cells. Undiluted whole blood is placed directly into a staining solution that labels cellular nucleic acid content and mitochondrial membrane potential (MMP). Samples are analyzed on a flow cytometer and the various cell types, including lymphocytes, reticulocytes and neutrophils, are differentiated based on light scatter profile and staining characteristics. To test the ability of HARM to detect radiation exposure, C57Bl6 mice were exposed to total body IR of up to 9 Gy via a ^{137}Cs source and peripheral blood was collected at various times post exposure. Data reveal varying time and dose-dependent sensitivities to IR in several populations of blood cells. Lymphocyte frequency was significantly reduced at earlier times and lower IR doses than reticulocytes or neutrophils. In addition to the effects on cell number, the indicator of MMP revealed that cell populations expressing a reduced frequency were also positive for an apoptotic signature. Administration of the radio-modifying agent amifostine to mice 30 min prior to IR revealed partial protection of reticulocytes from IR-induced damage/loss. HARM's simple methodology provides a multiparametric assessment of the effects of IR that could potentially be applied to human exposure assessment scenarios. This method could play an immediate role in responding to radiological incidents or as an enabling technology where additional endpoints relevant to radiation dose assessment are incorporated into the assay.

159 SIMULTANEOUS DETERMINATION OF CYANIDE, THIOCYANATE AND 2-AMINOTHIAZOLINE-4-CARBOXYLIC ACID (ATCA) BY HPLC.

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A method was developed for the quantitative determination of cyanide and its metabolites: ATCA and thiocyanate. Two distinct peaks were present for cyanide and thiocyanate after derivatization of cyanide. Two peaks were also present for ATCA, implying the possibility of simultaneous analysis of ATCA and the formation of 2-iminothiazolidine-4-carboxylic acid (ITCA), the tautomer of ATCA. The isolation and identification of these two compounds will be addressed. This research was funded under NIH/USAMRICD Interagency agreement Y1-A1-6176-01 via Contract under the auspices of the US Army Research Office Scientific Services Program administered by Battelle (Delivery Order 399, Contract No. DAAD19-02-D-001).

160 AEOL 10150 RESCUES THE LUNG FROM HALF-MUSTARD (2-CHLOROETHYL ETHYL SULFIDE)-INDUCED INJURY.

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Sulfur mustards (SM) have been used in warfare since WWI and could still pose a significant threat. SM gas exposure can cause significant blistering of the skin as well as respiratory injury and fibrosis. Currently there is no antidote for Mustard gas exposure and only symptomatic treatment is available. In the current study compounds were screened for potential protective effects against the SM analog 2-Chloroethyl ethyl sulfide (CEES) in an airway cell culture model. Lung epithelial cells, A549 and human small airway epithelial (SAE) cells, were treated with CEES for 48 hours to produce a significant reduction in cell viability. Of the screened compounds, a small catalytic antioxidant molecule, AEOL 10150, provided the most promising protection against CEES exposure. In addition, AEOL 10150 significantly rescued SAE cells from CEES exposure as measured by the retention of the fluorescent dye calcein. We then wanted to determine if toxicity was due, at least in part, to an increase in reactive oxygen species (ROS). Using MitoSox, a fluorescent indicator of ROS in the mitochondria, coupled with flow cytometry, ROS production was found to be increased 12 hrs after CEES exposure. AEOL 10150 was tested in vivo in Sprague-Dawley rats exposed nose-only to nebulized CEES (5% in ethanol for 30 min) and lung injury assessed 18 hrs later. AEOL 10150 (5 mg/kg, sc) was given 1 hr post CEES exposure and again 6 hrs later. Lung edema and hemorrhage was assessed by changes in the bronchoalveolar lavage protein and red blood cell levels. AEOL 10150 significantly attenuated SM gas-induced lung edema and hemorrhage. This suggests that AEOL 10150 rescues the lung from SM gas exposure and may provide a countermeasure against SM gas-induced lung injury. [Work was supported in part by NIH grant U54 ES 015678].

161 SURFACE ENHANCED RAMAN SPECTROSCOPY AS A FIELD SENSOR FOR THE DETECTION OF CYANIDE EXPOSURE.

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Cyanide is highly toxic and has been used as a chemical warfare agent in military operations. Rapid, precise and accurate on-site detection of cyanide has been a challenge for scientists, first responders and the military. Surface-Enhanced Raman spectroscopy (SERS) is a powerful technique, which uses metal surfaces to produce an enhancement of Raman scattering due to the adsorption of molecules to these surfaces. In the current study, cyanide and its metabolite thiocyanate were rapidly analyzed from human saliva of smokers, using SERS. Due to the portability, sensitivity, and speed of analysis (< 1 min), SERS could be used as a field sensor for rapid detection of cyanide exposure. This research was funded under NIH/USAMRICD Interagency agreement Y1-A1-6176-01 via Contract under the auspices of the US Army Research Office Scientific Services Program administered by Battelle (Delivery Order 399, Contract No. DAAD19-02-D-001).

162 STERICALLY STABILIZED LIPOSOMES ENCAPSULATING RHODANESE FOR CYANIDE ANTAGONISM.

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The major mechanism of removing cyanide (CN) from the body is its enzymatic conversion by a sulfurtransferase, e.g. rhodanese (Rh), to the less toxic thiocyanate in the presence of a sulfur donor (SD). Since Rh is a mitochondrial enzyme, and most of the SDs have limited cell penetration, the approach of placing a SD and exogenous Rh in a closer proximity by injecting them into the blood stream provides a significant increase in the in vivo protection with a given SD. Minimizing the immunologic reactions by encapsulating the enzyme is crucial for making this approach efficient in vivo. Earlier results proved the success of this approach with other carrier systems in the presence of various SDs in CN antagonism. Present studies are focusing on the liposomal carrier systems. Physicochemical properties e.g. membrane rigidity (electron paramagnetic resonance spectroscopy), size distribution (dynamic light scattering), osmolarity, and viscosity were determined for various liposomal lipid and hydrating buffer compositions to characterize the in vivo stabilities of the stealth liposomal carrier systems for the CN antidotal studies. For the in vitro kinetic studies with liposome encapsulated Rh and for determining encapsulation efficiencies with various liposomal compositions, the formation of thiocyanate was measured spectrophotometrically. The maximum encapsulation efficiency of 86% was achieved with the lipid composition of 57% palmitoyloleoylphosphatidylcholine and 58% cholesterol and 5% PEG2000-dipalmitoylphosphatidylethanolamine with a hydrating buffer of HEPES.

163 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN-MEDIATED IMPAIRMENT OF B CELL DIFFERENTIATION THROUGH DEREGULATION OF PAIRED BOX GENE 5 (PAX5).

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The persistent environmental contaminant, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), is a potent suppressor of humoral immune responses. TCDD acts directly on B cells through a mechanism involving the ligand-activated transcription factor, the aryl hydrocarbon receptor (AHR). The B cell differentiation repressor, Pax5, has been implicated in the suppression of the immunoglobulin M (IgM) response to bacterial lipopolysaccharide (LPS) in the murine B cell lymphoma, CH12.LX, by TCDD. We proposed that this suppression of the IgM response in

LPS-activated CH12.LX cells by TCDD occurs due to the impairment of terminal differentiation by a mechanism involving deregulation of Pax5. The full-length isoform, Pax5a, and at least two additional Pax5 isoforms were detected in CH12.LX cells, all of which were abnormally elevated in LPS-activated cells treated with TCDD. Concordantly, Pax5 protein levels were down-regulated post LPS-activation, but remained abnormally elevated post LPS and TCDD co-treatment. Furthermore, B cell differentiation markers XBP-1 and MHC class II were altered by TCDD in agreement with the elevation in Pax5. Confirming the involvement of Pax5, ectopic expression of Pax5a in the LPS-activated CH12.LX cells closely mimicked the suppression of the IgM response by TCDD. In summary, our results demonstrate a critical role by Pax5a in the impairment of B cell differentiation and the suppression of the humoral immune response by TCDD. (Supported in part by NIH ES02520 and T32 ES07255).

164 A COX-2 METABOLITE OF 2-ARACHIDONYL GLYCEROL IS INVOLVED IN THE SUPPRESSION OF IL-2 SECRETION IN ACTIVATED JURKAT T CELLS.

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Previous studies from our laboratory have demonstrated that 2-arachidonyl glycerol, an endocannabinoid, suppresses IL-2 secretion in activated T cells through PPARγ activation independent of the cannabinoid receptors, CB1 and CB2. Many COX-2 products have been demonstrated to activate PPARγ. Owing to the structural similarity between ligands of PPARγ and COX-2 metabolites of 2-AG, the role of COX-2 metabolism in the 2-AG-mediated suppression of IL-2 secretion was investigated. Pretreatment with non-selective and COX-2 specific inhibitors completely abolished 2-AG mediated suppression of IL-2 secretion, whereas pretreatment with COX-1 specific inhibitors had no effect on 2-AG-mediated suppression of IL-2 secretion. Western blot analyses of Jurkat T cells and murine splenocytes demonstrated that COX-2 protein is readily detectable in resting Jurkat T cells, whereas in resting murine splenocytes there is virtually no basal expression of COX-2 protein. In addition, COX-2 protein and mRNA levels are markedly upregulated by 2h following activation of Jurkat T cells, whereas in murine splenocytes, the upregulation of COX-2 protein was observed at and after 4h following activation. COX-2 metabolism of 2-AG may be responsible for the production of ligands for PPARγ activation, which may ultimately result in the suppression of IL-2 secretion. The differential expression of COX-2 protein in various T cell models may explain the varied potency of several endocannabinoids in the suppression of IL-2 secretion. (Support in part by NIH RO1 DA12740).

165 ROLE OF NF-κB/REL PROTEINS IN MEDIATING THE REPRESSIVE EFFECTS OF TCDD ON 3'IgHRR ACTIVATION.

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Transcriptional regulation of the immunoglobulin heavy chain (IgH) gene involves several regulatory elements including the 3'IgH regulatory region (3'IgHRR). The 3'IgHRR is composed of four distinct enhancers (hs3A, hs1,2, hs3B, hs4) and contains DNA binding sites for several transcription factors including NF-κB/Rel proteins. A recently identified DNA binding motif the dioxin responsive element (DRE) may also contribute to 3'IgHRR regulation. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), a known disrupter of B cell differentiation, induces binding of the aryl hydrocarbon receptor (AhR) nuclear complex to a DRE motif within the hs1,2 and hs4 enhancers. Interestingly, TCDD profoundly inhibits the activation of the 3'IgHRR by the B cell activator lipopolysaccharide (LPS) but enhances LPS-induced activation of the hs4 enhancer. Within the hs4 enhancer, the DRE overlaps an NF-κB/Rel binding motif (κB) and site directed mutational analysis demonstrated a cooperative interaction between proteins binding to these motifs. The objective of the current studies was to evaluate the expression of NF-κB/Rel proteins including their negative regulator IκBα following LPS stimulation and TCDD treatment and to compare these results to 3'IgHRR activity under the same treatment conditions. For these studies we utilized the CH12.LX B cell line and its variant, CH12.IκBαAA, which expresses an inducible IκBα super repressor (IκBαAA). Western blot analysis demonstrated a marked decrease in endogenous IκBα with a reciprocal increase in nuclear RelA in the CH12.LX cells 1 hr after LPS stimulation. In the CH12.IκBαAA cells endogenous IκBα was also decreased by 1 hr after LPS stimulation. However, maximal expression of the IκBαAA transgene could be induced by 1 hr and expression of the IκBαAA partially attenuated both LPS-induced activation of the 3'IgHRR and hs4 activation following an LPS and TCDD co-treatment. These results suggest that NF-κB/Rel proteins are at least partially responsible for 3'IgHRR modulation by LPS and TCDD.(Supported by Boonshoft School of Medicine, WSU and NIEHS R01ES014676)

166 3, 4-DICHLOROPROPANANILIDE ALTERS T CELL ACTIVATION BY INHIBITING THE CA2+ INFLUX PATHWAY.

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Stimulation through the T cell receptor (TCR) results in the activation of a series of signaling pathways leading to IL-2 secretion and proliferation of the T cell. One of the early steps in the activation process is the release of calcium (Ca2+) from internal stores coupled to an influx of Ca2+ from the extracellular environment. Ca2+ release-activated Ca2+ (CRAC) channels are store-operated Ca2+ channels that are activated upon internal Ca2+ store depletion and provide the elevated and sustained intracellular calcium concentration ([Ca2+]i) required for optimal T cell activation. Alterations in CRAC channel activity result in decreased [Ca2+]i and reduce or prevent T cell activation. DCPA (3,4-dichloropropananilide) is a common herbicide used against broad leaf vegetation in rice and wheat fields. The effect of DCPA on mammalian immune cells has been well documented and include a reduction of IL-2 production in T cells. Three important transcription factors involved in IL-2 production, NFAT, NF-κB and AP-1, are altered in DCPA-treated T cells and all 3 depend, to varying degrees, on Ca2+ dependent signaling events. Due to the importance of Ca2+ homeostasis in T cell activation and function, we investigated the effect of DCPA on [Ca2+]i in T cells. Our experiments indicate that DCPA may inhibit T cell activation through alterations in the Ca2+ influx pathway. Supported by NIH ES011311 and EPA Fellowship MA91684801.

167 TCDD-INDUCED MODULATION OF THE HUMAN POLYMORPHIC HS1, 2 ENHANCER WITHIN THE 3'IgH REGULATORY REGION.

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Transcriptional regulation of the immunoglobulin heavy chain (IgH) gene involves several regulatory elements including the 3'IgH regulatory region (3'IgHRR). The 3'IgHRR is composed of four enhancers (hs3a, hs1,2, hs3b, hs4) and contains DNA binding sites for several transcription factors including NF-κB/Rel proteins. The dioxin responsive element (DRE) may also contribute to the regulation of the 3'IgHRR. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a known disrupter of B cell differentiation, induces binding of the aryl hydrocarbon receptor (AhR) nuclear complex to DRE motifs within the hs1,2 and hs4 enhancers and produces a marked inhibition of 3'IgHRR activation, IgH expression, and antibody secretion in a well-characterized mouse B cell line (CH12.LX). Interestingly, in humans a polymorphism of the hs1,2 enhancer (resulting in a varying number of tandem repeats of a 53 bp sequence) has been correlated with severe IgA nephropathy, the most common glomerular disease worldwide. The repeated sequence contains a κB and DRE binding site. The purpose of this study was to evaluate the effects of TCDD and lipopolysaccharide (LPS) stimulation on the activity of the polymorphic human hs1,2 enhancer in our CH12.LX cell line model. As might be expected, an increased number of tandem repeats increased the sensitivity to LPS activation. Interestingly, TCDD activated the human hs1,2 enhancer in the absence of LPS stimulation and an increased number of tandem repeats increased the magnitude of TCDD-induced activation. However, previous results have demonstrated a TCDD-induced inhibition of the mouse 3'IgHRR but activation of the hs4 enhancer. These results suggest a difference in transcriptional regulation between the mouse and human hs1,2 sequence. Chemical-induced modulation of the hs1,2 polymorphism may provide an environmental clue to the etiology of IgA nephropathy. (Supported by the Boonshoft School of Medicine, WSU, and NIEHS R01ES014676)

168 EFFECT OF TCDD ON THE TRANSCRIPTIONAL ACTIVITY OF THE 3'IgHRR ENHANCER ELEMENTS HS3A AND HS1, 2.

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2,3,7,8,-tetrachlorodibenzo-p-dioxin (TCDD) is a potent environmental toxin that inhibits immunoglobulin (Ig) gene expression. Transcriptional regulation of the Ig heavy chain (IgH) involves the 3'IgH regulatory region and its four enhancers

(hs3A, hs1,2, hs3B, and hs4) that have DNA binding sites for several transcription factors, including NF-κB. Additionally, a dioxin responsive element (DRE) has been identified in hs1,2 and hs4. TCDD also produces a marked inhibition of the 3'IgHRR in B cells stimulated with LPS but also triggers a profound activation of the hs4 enhancer in LPS-activated cells. Because this hs4 activation contradicts the inhibitory effect of TCDD on the 3'IgHRR, the objective of the current study was to determine if the hs1,2 enhancer mediates the inhibitory effect of TCDD on 3'IgHRR activity. From the well-characterized CH12.LX mouse B cell line, we developed the CH12.γ2b-3'IgH cell line, which expresses an inducible γ2b transgene under the regulation of the 3'IgHRR with loxP sites flanking the hs3B/hs4 enhancer pair. Expression of the γ2b transgene is sensitive to LPS activation and TCDD inhibition as seen with transient 3'IgHRR luciferase studies. Through real-time PCR, the CH12.γ2b-3'IgH cells were found to have one copy of the γ2b transgene. To induce deletion of the hs3B/hs4 enhancer pair through CRE/LoxP-mediated DNA recombination, the CH12.γ2b-3'IgH cells were transiently transfected with a CRE-GFP plasmid. Cells expressing GFP were selected and subcloned. Studies are ongoing to test the clones for CRE-mediated deletion of the hs3b/hs4 enhancers. Verified deletion derivatives will be compared to the parental line to determine the concentration-dependent effect of TCDD on γ2b expression in LPS-stimulated B cells. Evaluating the relative importance of each enhancer pair in mediating the effects of B cell stimulants and TCDD on 3'IgHRR activity will provide the foundation for future studies aimed at identifying the molecular components involved in 3'IgHRR and IgH gene regulation. (Supported by the Boonshoft School of Medicine, WSU, and NIEHS R01ES014676)

169 EVALUATION OF POSSIBLE MODES OF ACTION FOR PFOS-INDUCED HUMORAL IMMUNOSUPPRESSION.

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Perfluorinated hydrocarbons have been manufactured for over 40 years and have numerous applications in industry. This group of compounds has recently generated much interest as some of these compounds (i.e., perfluorooctane sulfonate (PFOS)) are persistent in the environment and are detectable in blood samples of both wildlife and humans. Studies show that these perfluorinated compounds cause various toxicological effects; however, effects on immune function have not been addressed at length. We have previously shown decreased SRBC-specific IgM production in adult B6C3F1 mice following exposure to PFOS. The current study assessed possible modes of action for this suppression. Adult female B6C3F1 mice were exposed orally to PFOS for 28 days. Numbers of B-cells were not decreased in the spleen suggesting that simple depletion of B-cells (CD19+ or CD21+) is not responsible for the decrease in IgM production. Numbers of MHCII+ and CD40+ cells were also not altered. Expression of CD154 (CD40L) did not differ between treatment groups and control. Anti-CD40- and LPS-induced IL-6 production by B-cells was increased. IL-4, IL-5 and IL-6 production by T-cells (anti-CD3 and PMA-induced) was not effected. However, suppression of TNP-specific IgM occurred following challenge with TNP-LPS. This indicates that the observed suppression of humoral immunity may be related to B-cell function as IgM production is suppressed by both T-dependent (SRBC) and T-independent (LPS) antigens.

170 DRUG METABOLISM AND IMMUNE RESPONSE CROSSTALK: THE ROLE OF PREGNANE X RECEPTOR IN MHC-II TRANSCRIPTIONAL REGULATION.

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Pregnane X Receptor (PXR) is known to act as a sensor to a broad range of natural and synthetic xenobiotics to mediate the induction of CYP3A detoxification enzyme as well as through other drug metabolizing enzymes. Recently, the roles of PXR in other transcriptional regulation and its interaction with CREB have been demonstrated. Adding to these findings, activation of rodent PXR has been shown to induce mRNA and protein levels of Major Histocompatibility Complex class II (MHC-II) in primary hepatocytes. The role of PXR and its possible involvement in the immune response has triggered further research. To investigate further the molecular mechanism by which PXR induces transcription of MHC-II genes, we performed a dose response experiments with PXR activator at different time courses in human bronchial epithelial cells (BEAS-2B). To investigate any crosstalk between PXR and the immune response, we also performed a dose response experiments with IFN gamma to evaluate changes in PXR mRNA levels. Preliminary results showed 3.2 folds and 8.9 folds induction of HLA-DR alpha gene in BEAS-2B cells after 4hr treatment with rifampicin and IFN gamma, respectively. The presence of PXR mRNA this cell line was confirmed by means of RT-PCR and showed a 2 folds and 3.6 folds induction of PXR mRNA levels after 4hr treatment with rifampicin and IFN gamma, respectively. Preliminary results also showed a dose response in the PXR mRNA levels after IFN gamma treatment. PXR and CYP3A4 mRNA expression was found to be induced in BEAS-2B cells treated for 24hr with IFN gamma. MHC-II gene expression was also induced in cells treated with the PXR activator, SR-13128. These findings support the hypothesis of a potential involvement of PXR by enhancing transcriptional induction of MHC-II genes. These results continue adding a new dimension to a relationship of immune and detoxification regulation through PXR function as means of a xeno-protective mechanism.

171 METALLOTHIONEIN GENE DOSE INFLUENCES CYTOKINE SECRETION PROFILE.

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Metallothionein (MT) is a small cysteine rich stress response protein associated with zinc homeostasis and metal detoxification. It also plays an important role in scavenging reactive oxygen species. Previous studies have shown that MT is immunomodulatory: exogenous MT has a suppressive effect on T-cell dependent humoral immunity, splenocytes from mice with disrupted Mt genes exhibit enhanced T-dependent humoral immunity, and mice treated with an anti-MT monoclonal antibody also exhibit enhanced T-dependent humoral immunity. To better understand the role of MT in immune response, we used three C57BL/6J congenic mouse strains: MT WT, MT TgN), and MT KO. Challenged with ovalbumin (OVA) in the presence or absence of cadmium (Cd) or zinc (Zn) exposure, KO mice exhibited increased serum IgG1 anti-OVA compared to WT controls. The dose of Cd and Zn we chose did not change the T-dependent anti-OVA humoral response in the WT mice, however, this same dose of both Cd and Zn caused significant immunosuppresion in the TgN mice, while only the Cd was immunosuppressive in the KO mice. To further explore how MT gene dose influences anti-OVA response, we stimulated splenocytes with PMA/ionomycin and measured cytokines in the supernatants. KO splenocytes produce high levels of IL-4 compared to both WT and TgN splenocytes, WT splenocytes produce higher levels of IFN-γ compared to KO and TgN splenocytes and higher levels of IL-2 compared to TgN splenocytes. This data indicates that KO mice express a Th2 cytokine profile that favors B cell class switching to the IgG1 producing plasma cells. (Supported by NIEHS grant # ES07408)

172 MECHANISMS OF DITHIOCARBAMATE IMMUNOMODULATION: ALTERED CELLULAR REDOX STATUS.

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Sodium methyldithiocarbamate (SMD) is the third most abundantly used pesticide in the U.S., and exposure to levels above those recommended as safe by the EPA occurs to hundreds of thousands of people. This compound decreases the survival of mice challenged with a non-pathogenic Escherichia coli, a model for sepsis associated with a ruptured appendix or other abdominal trauma, but its mechanism of action is not known. Reduced, oxidized, and total glutathione in peritoneal macrophages was measured 1-4 hr after treatment with SMD (100-300 mg/kg intranasally). SMD alone or with LPS caused a substantial decrease in reduced and total glutathione. Oxidative stress was further demonstrated by analyzing macrophages from an SMD dose response experiment by microarray (Affymetrix). Substantial upregulation was observed for genes associated with protection from oxidative stress (e.g., catalase, superoxide dismutase, and GSH synthase). Treatment of mice with N-acetyl cysteine (NAC, to increase GSH synthesis) or buthionine sulfoximine (BSO) to deplete GSH had essentially opposite effects. For example, IL-12 production was inhibited by SMD and further inhibited by SMD + NAC. In contrast, BSO essentially reversed the effects of SMD on IL-12 production. Thus, SMD decreases reduced glutathione and upregulates genes associated with protection from reactive oxygen. These findings seem to suggest that SMD acts on cytokine production by increases reactive oxygen species. However, further depletion with BSO actually prevents suppression of IL-12 production by SMD, and NAC + SMD causes ever greater suppression of IL-12. This would suggest that more reactive oxygen favors IL-12 production and less favors inhibition. However, increased reactive oxygen alone cannot account for the effects, because BSO does not suppress IL-12 production. It seems likely that redox potential regulates effects caused by another mechanism, probably chelation of copper by SMD. This work was supported by NIEHS grant R01ES013708-02.

173 ALTERATIONS IN POLY I:C-INDUCED CYTOKINE PRODUCTION BY ACUTE ETHANOL ADMINISTRATION: ROLE OF NEUROENDOCRINE MEDIATORS.

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Acute high dose ethanol exposure has been associated with many immunosuppressive effects, including a reduction in pro-inflammatory cytokine production. Studies have indicated that some of these effects are mediated, at least in part, by the increase in glucocorticoids (corticosterone in mice). However, the sympathetic nervous system (SNS) is also activated in stress situations, resulting in the release of catecholamines. The relative importance of glucocorticoids and catecholamines is not clear, and this study was designed to investigate this issue. Nadolol (beta adrenergic antagonist), corticosterone, or placebo pellets were implanted subcutaneously in adrenalectomized (ADX) mice. One day later, mice were treated with poly I:C (100 μg/ml, I.V.) with or without acute ethanol (6g/kg, oral gavage), and serum samples were tested for corticosterone (by EIA), cytokines, and chemokines (by multiplexed bead array assay or ELISA). In general, no suppression of cytokines or chemokines via catecholamines was demonstrated at this time point (2 hours after poly I:C). Some glucocorticoid suppression of pro-inflammatory cytokines (e.g. IL-6 and IL-12) was evident with poly I:C stimulation when comparing adrenalectomized mice and adrenalectomized mice with a corticosterone pellet yielding normal or greater than normal corticosterone levels. However, further suppression of pro-inflammatory cytokines by the administration of acute ethanol did not appear to be attributable to corticosterone or catecholamines, because the effects of ethanol were not reversed by adrenalectomy or nadolol pellets. Further studies at earlier time points will be performed to determine whether catecholamines or glucocorticoids play a role in the cytokine alterations demonstrated with acute ethanol administration, and if so, at which point in the pathway. This work was supported by NIH grant AA009505.

174 IMMUNE FUNCTION IN INTACT AND PPARα KNOCKOUT MICE EXPOSED TO PFOA.

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Perfluorooctanoic acid (PFOA), used in fluoropolymer production, is environmentally persistent, present in human and wildlife populations worldwide, and associated with health effects in laboratory animals, including immunomodulation. PFOA toxicity may be mediated by the peroxisome proliferator activated receptor alpha (PPARα). Lymphoid tissues of mice deficient in PPARα (KO) were reported to be less susceptible to PFOA than wild-type (WT) mice. We therefore exposed Sv129 or C57BL/6 PPARα KO and WT mice to 0-30 mg PFOA/kg BW in drinking water for 15d. On d11 of dosing, mice were i.v. immunized with sheep red blood cells (SRBCs). Serum for evaluation of IgM titers was collected 1d post-dosing (PD). Booster immunizations were given 10d PD; serum for evaluation of IgG titers was collected 15d PD. In Sv129 mice, IgG titers were similar in dosed and control groups in Sv129s; IgM titers were statistically elevated in KOs (30 mg/kg) relative to controls and unaffected in WTs. In C57BL/6 mice, IgM titers were statistically suppressed in WTs and KOs exposed to 30 mg/kg relative to controls; IgG titers were not altered. PFOA did not alter lymphoid organ weights in WT or KO Sv129 or KO C57BL/6 mice; however, spleen weights were statistically reduced in C57BL/6 WTs, when evaluated 1d PD. Absolute and relative liver weights were statistically increased in all exposed groups. Sensitivity to PFOA, based on liver weight increases, was Sv129 WT > Sv129 KO > C57BL/6 WT > C57BL/6 KO. However, based on alteration of IgM titers, sensitivity to PFOA was C57BL/6 WT > C57BL/6 KO > Sv129 KO > Sv129 WT. This difference suggests that strain sensitivity to PFOA and non-PPARα mechanisms influence liver weights and antibody synthesis. In a separate study, IgM titers were statistically reduced in adrenalectomized C57BL/6 WTs relative to controls, thus minimizing a central role for stress-induced corticosterone release as the prime driver for reduced antibody synthesis. (This abstract does not reflect EPA policy and was supported by UNC/EPA Cooperative Training Agreement CT829472.)

175 HISTOLOGIC FEATURES OF THE DEVELOPING IMMUNE SYSTEM OF RATS.

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There is some evidence that the immature immune system is more susceptible to chemically mediated perturbations, or may show responses to chemical exposure that are different from the responses of the adult immune system. It has been proposed that safety assessment studies should include investigation of immune system alterations in neonatal or immature animals, possibly as an adjunct to existing developmental and reproductive toxicology (DART) protocols. Histopathologic evaluation of immune system organs and tissues would be an important endpoint of these investigations. These evaluations will require an in-depth knowledge of the normal microscopic features of immune system organs and tissues, which are poorly characterized at present. The purpose of the present study was to perform standard histopathologic examination of immune system organs collected from rats at the later stages of gestation and through the early postnatal period. Specimens of thymus, spleen, lymph nodes, bone marrow, lung and intestinal tract of rats from gestation day 15 (GD15) through post-natal day 42 (PND42) were fixed in formalin, subjected to standard histologic processing, and examined via light microscopy. Results suggest that immune system organs are not sufficiently developed at GD15 for meaningful histopathologic evaluation. At GD20 the major organs of the immune system were identifiable, but very immature. Immune system organs at PND22 are histologically similar to those of adult rats, but have little indication of immunologic stimulation.

176 EFFECTS OF GLUTATHIONE DEPLETION ON PULMONARY AND SPLENIC INFLAMMATORY CELLS FOLLOWING EXPOSURE TO DIESEL EXHAUST.

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Exposure to particulate matter (PM), a major component of air pollution, has been associated with cardiorespiratory morbidity and mortality. While the exact mechanisms are unclear, these adverse events are likely mediated by inflammation and oxidative stress, both of which are characterized in part by deregulated immune responses. Oxidative stress can be attenuated by many antioxidants, including the tripeptide thiol glutathione (GSH). GSH biosynthesis is rate-limited by the enzyme glutamate-cysteine ligase (GCL), which is composed of catalytic (GCLC) and modifier (GCLM) subunits. Our laboratory has generated GCLM-null mice, which have approximately 80% lower GSH levels than their wild-type littermates. GCLM-null mice are very sensitive to the toxic effects of agents that utilize GSH-dependent detoxification. However, the immune systems of these mice have not been characterized following exposure to oxidants. To examine the effects of PM, specifically diesel exhaust (DE) on oxidative stress and inflammation, GCLM wild-type and null mice were exposed to DE at 0, 200 and 400 $\mu g/m^3$ for 3 days (6 hr per day). Following sacrifice, the percent and number of macrophages, neutrophils and lymphocytes were determined in the bronchoalveolar lavage and spleen. Exposure to high levels of DE decreased the percentage of neutrophils in the spleens of both wild-type and GCLM-null mice. Furthermore, diminished GSH levels correlated with fewer $CD4^+$ T cells in the spleens of GCLM-null mice following 400 $\mu g/m^3$ DE exposure. The percent and number of $CD4^+$ and $CD8^+$ T cells, neutrophils and macrophages in lung lavage were not altered following DE exposure using this regimen. These studies are ongoing and inflammatory cytokine levels in the plasma are currently being evaluated. At this time, our preliminary data show that acute exposure to DE has minimal effects on immune cell numbers in the lung and spleen, regardless of GCL status.

177 IMMUNOTOXIC EFFECTS AFTER UTERO AND LACTATIONAL EXPOSURE TO ENDOSULFAN IN LYMPH NODES, SPLEEN AND THYMUS OF IMMATURE RATS.

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Endosulfan is an organochlorine insecticide, considered as neuroendocrine disrupter. This pesticide induces immunotoxic effects, although its immunotoxicity is poorly studied. Like other organochlorines, endosulfan accumulates in fatty tissue, and it can cross the placenta arriving to the foetus and breeding after its birth through maternal milk. The objective of the present work was to evaluate possible immune alterations induced by endosulfan exposure during pregnancy and lactation in offspring rats. The pesticide was administered to mothers orally at two doses (0.61 and 6.12 mg endosulfan/kg/day). Offspring were sacrificed at 30th day of life. Different lymphocytes populations and subsets were analyzed by flow immunocytometry in submaxillary lymph nodes, spleen and thymus. The two doses of endosulfan administered increased the percentage of B+ lymphocytes in lymph node of male and female offspring, and increased the percentage of T+, CD4+ and CD8+ cells in this lymphoid organ, but only in females. In spleen, the dose of 6.12 mg/kg/day induced an increase of the percentage of B+ lymphocytes in both male and female offspring and a decrease in T+ and CD4+ lymphocyte percentage in

males; while the lowest dose administered decreased T+ lymphocytes in male offspring. The percentage of B+ cells showed an increase in thymus of male and female rats after endosulfan exposure at the highest dose, and the two doses studied induced a decrease of the percentage of CD8+ lymphocyte. These results suggest that oral endosulfan exposure during pregnancy and lactation can induce immunotoxicity in male and female offspring, with effects on cellular and humoral immunity. This work was supported by a grant from the Ministry of Education and Science, Spain (AGL2004-04543/ALI).

178 METALLOTHIONEIN EXPRESSION AFFECTS THE INNATE IMMUNE RESPONSE TO INFECTION.

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Metallothioneins (MTs) are small cysteine-rich proteins with a wide range of functions including reactive oxygen species (ROS) scavenging, chemotactic activity, immunomodulation, essential cation management and toxic metal sequestration. MT-I and MT-II are the most widely studied isoforms and are highly conserved among mammals. Listeria monocytogenes is a Gram-positive bacterium that can be pathogenic in humans and is commonly used as a model of mammalian immune response to infection. We have used an in vivo model of listeriosis to evaluate the effects of MT gene dose on the course of Listeria infection in three strains of mice: C57BL/6 wild-type mice (WT), congenic MT-I/II knock-out mice (MTKO), and congenic MT-I transgenic mice (MTTG). Our results suggest that either overexpression of MT-I or a lack of functional MT-I and MT-II will significantly alter the course of intravenous listeriosis. Both MTTG and MTKO mice show significantly lower Listeria burdens in liver and spleen three days post-inoculation. A time course study suggested that this difference is most prominent in the first 48 hours of infection, during the innate immune response, after which Listeria clearance occurs at comparable rates in all three strains. DHR fluorescence assays revealed that lymphocytes from the MTTG mice exhibit an increased oxidative burst compared to WT and MTKO strains. In vivo experiments reveal that Listeria infection results in decreased IL-6 levels in liver and spleen and decreased TNF-α levels in spleen in both MTTG and MTKO mice compared to WT. Mitogen stimulation results in lower IL-2 and IFN-γ secretion from MTKO and MTTG splenocytes compared to WT splenocytes. Macrophages from MTTG and MTKO mice exhibit an increased oxidative burst compared to WT macrophages, suggesting a possible mechanism by which these strains experience a lower Listeria burden than WT mice. Our results indicate that MT levels can dramatically influence the innate immune response to infection. (Supported by NIH grant ES #07408)

179 EXPOSURE TO ENVIRONMENTAL BACTERIA IS ASSOCIATED WITH TNF-ALPHA AND IL-6 PRODUCING CAPACITY IN INFANCY.

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There is increasing evidence suggesting that early life exposure to environmental microbes and their compounds may play a crucial role in maturation of the immune system. In the present study, we aimed to investigate associations between stimulated proinflammatory cytokine responses in neonatal and maternal blood samples and concentrations of microbial markers in house dust samples. Peripheral blood samples of children (n=24) and their mothers (n=25) were collected 3 months after birth, stimulated for 8 hours with the combination of PMA and ConA, and analyzed for detection of IL-6 and TNF-α using enzyme-linked immuno sorbent assay. Gas chromatography mass spectrometry was used to quantify the concentrations of microbial markers (ergosterol, fungi; muramic acid, mainly Gram-positive bacteria; 3-hydroxy fatty acids, Gram-negative bacteria) in house dust samples. A written informed consent was obtained from all subjects and the study protocol was approved by the Research Ethics Committee, Hospital District of Northern Savo. We found an inverse association between concentration of 3-OH fatty acids (C10 to C14) in bed dust and the production of TNF-α and IL-6 in both mothers and children. In contrast, high concentration of muramic acid in floor dust was related to increased production of TNF-α and IL-6. In conclusion, exposure to Gram-negative bacteria may be associated with the development of immunotolerance already in infancy, indicated as an impaired production of proinflammatory cytokines in response to mitogen-stimulation. Gram-positive bacteria seem to have opposite effects. These findings suggest that different microbes may have distinct effects on the maturation of immune system.

180 DICHLORVOS SIGNIFICANTLY DECREASES THE EXPRESSION OF GRANZYME B AND GRANZYME 3/K IN HUMAN NK CELLS.

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Natural killer (NK), lymphokine-activated killer (LAK), and cytotoxic T lymphocyte (CTL) cells kill target cells by the directed release of cytolytic granules that contain perforin, granzymes, and granulysin. We have found previously that dimethyl 2,2-dichlorovinyl phosphate (DDVP, dichlorvos), an organophosphorus pesticide, significantly decreased the expression of perforin, granzyme A (GrA), and granulysin and inhibited NK, LAK, and CTL activities. To further explore the mechanism of organophosphorus pesticide-induced inhibition of cell-mediated cytolysis, we examined whether organophosphorus pesticides affect the expression of GrB and Gr3/K in NK cells. We used an interleukin-2 (IL-2) independent human NK cell line, NK-92CI. We confirmed that NK-92CI cells express CD56 surface marker, intracellular perforin, GrA, GrB, Gr3/K, and granulysin by flow cytometry, and that NK-92CI cells are highly cytotoxic to K562 cells with a chromium release assay. NK-92CI cells were incubated with DDVP at 0, 0.028, 0.055, 0.110, 0.225, and 0.450 mM for 4, 15, and 24 h at 37°C in a 5% CO2 incubator, then harvested, washed with PBS. Intracellular levels of GrB and Gr3/K in NK-92CI cells were determined by flow cytometry and NK activity was measured with a chromium release assay. We confirmed that DDVP significantly inhibited NK activity in a dose-dependent manner. Then, we found that DDVP significantly decreased the expression of GrB and Gr3/K in NK-92CI cells in a dose- and time-dependent manner. Immunocytochemical results showed that DDVP significantly decreases the level of GrB positive granules in NK-92CI cells, which may be due to degranulation. Taken together, DDVP significantly inhibits NK activity, which is at least partially mediated by the reduction of the intracellular GrB and Gr3/K levels in NK cells.
This work was supported by grants from the Ministry of Education, Culture, Sports, Science, and Technology (No. 15590523 and No. 19590602).

181 PRENATAL EXPOSURE TO CIGARETTE SMOKE SUPPRESSES CYTOTOXIC T-LYMPHOCYTE (CTL) ACTIVITY IN THE OFFSPRING POSSIBLY *VIA* INCREASED NUMBERS OF T-REGULATORY CELLS.

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In previous studies performed in this laboratory, we demonstrated that male mice, exposed prenatally to mainstream cigarette smoke (MCS) and then injected at 5 wk with lymphoma cells, had a >2-fold increase in tumor incidence than air-exposed controls. This effect appeared to be due to a concomitant suppression of cytotoxic T-lymphocyte (CTL) activity during tumor development. To determine the mechanism(s) by which CTL activity may have been reduced by fetal smoke exposure, 5- and 10-wk-old male offspring, exposed *in utero* to MCS, were challenged with EL4 lymphoma cells, the spleen and thymus recovered at 1, 8, and 15 d after injection, and cell types quantified and characterized by expression of CD3, CD4, CD8, B220, NK, CD25, CD11b, CD11c, HSA, and FOXP3 using flow cytometry. Results demonstrated that at 1 and 8 d post-tumor cell injection, the percentage of thymic $CD4^+CD25^+FoxP3^+$ regulatory (T_{reg}) cells was modestly increased (compared to age-matched air controls) in the 5-wk-old smoke-exposed offspring. A significant increased in T_{reg} cells was observed in the older smoke-exposed pups at d 15. At 8 d post-tumor challenge, prenatal exposure to CS significantly increased the percentage of splenic T_{reg} cells, and decreased the percentage of $CD8^+$ cells (among total splenic lymphocytes) in the 5-wk-old pups resulting in an increased CD4/CD8 ratio. Also at d 8, there was an increase in the mature CD4/CD8 ratio in the $CD3^+$ cell population due to an increase in $CD4^+$ cells. Given that T_{reg} cells have been shown to suppress activation and proliferation of $CD8^+$ T cells especially in the anti-tumor response, and the temporal relationship between effects on CTL activity and T_{reg} numbers, these findings suggest a possible mechanism by which prenatal exposure to CS may result in reduced CTL activity during tumor development. Supported by Philip Morris and Novartis Graduate Fellowship.

182 COMPARISON OF 28S RRNA CLEAVAGE BY RICIN AND TRICHOTHECENE DEOXYNIVALENOL IN RAW 264.7 MACROPHAGES.

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Both type II ribosome inactivating proteins (eg. ricin) and sesquiterpenoid trichothecenes (eg. deoxynivalenol [DON]) bind ribosomes, inhibit translation and activate mitogen-activated protein kinases (MAPKs). Here we compared the capac-

ities of ricin and DON to cleave 28S ribosomal RNA (rRNA). In a cell-free model, exposure to ricin with concentration of 300ng/ml for 30 min depurinated yeast 28S rRNA at 330 nucleotides (nt) from 3' terminal end. In contrast, DON at ≤4000 ng/ml did not directly depurinate yeast 28S rRNA. Incubation of RAW 264.7 macrophages with either 20 to 320ng/ml of ricin or 250 to 5000 ng/ml of DON for 6 h generated 28S rRNA-specific products consistent with cleavage sites 1.2 and 1.5 kilonucleotide (knt) from the 3' terminal end of mouse 28S rRNA. When ricin-treated RAW 264.7 cells were analyzed by primer extension, two cleavage sites were identified, one (A4325) at the ricin/sarcin loop, and another (C4037) at the center of the peptidyl transferase. Although similar DON exposure did not damage the ricin/sarcin loop, the treatment caused cleavage of 28S rRNA at two sites (C4037 and A3560) in the peptidyl transferase center, where anisomycin is known to act, and at a third site (G3656). RNase activity as well as RNase 6 and RNase L mRNAs were increased in RAW 264.7 cells that were incubated with ricin (≥ 20 ng/ml) or DON (≥125 ng/ml). These data suggest that ricin cleaves 28S rRNA directly and indirectly via RNase induction whereas DON induces 28S rRNA cleavage only by indirect induction of RNases.

183 TOXICOLOGICAL EFFECTS OF NAPHTHALENE ON INNATE IMMUNITY OF FISH (CENTROPOMUS PARALLELUS). EVALUATION BY FLOW CYTOMETRY.

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Flow cytometry is a precise, fast, and effective method for the evaluation of several cellular parameters, both structural and functional, allowing the sorting and analysis of particular populations and sub-populations of cells. In this study, we employed cytometric methods on the caractherization of different blood cell types from the fish Centropomus parallelus, and also verified cell viability, phagocytic activity and oxidative burst in these cells. Three sub-populations were identified: lymphocytes, monocytes, and trombocytes. These cells were stimulated in vitro with propidium iodide-conjugated Staphylococcus aureus (SAPI), escherichia coli lipopolysaccharide (LPS), or zymosan (Saccharomyces cerevisae particles). Responses to each individual stimulus differed according to cell type. Monocytes displayed the higher percentages of phagocytosis in presence of SAPI (83,2% of control group) or zymosan (66,3%); on the other hand, the population that included trombocytes, among other cell types (such as lymphocytes) only performed phagocytosis in a relevant level (fluorescence) in the presence of SAPI. The oxidative burst, detected by fluorescence emitted by 2,7,dichlorofluorescein diacetate (DCFH) was significant only after stimulation with PMA (phorbol myristate-acetate), but not when the stimulus was SAPI or LPS. Differents concentrations of naphthalene in vitro increased de porcentages of manocytes and trombocytes performed phagocytosis (18, 9 and 23,5% of controle group respective). Based on these preliminary results, the innate immune response in these fish (ubiquitous coastal waters of the Brazilian shore) can be further evaluated. Immunophysiological parameters in these species can build a solid ground for future ecotoxicological studies. Approaching innate immunity as a biological indicator of environmental changes induced by pollutants may support the degree of toxicological impact over these animals, and aggregate value to its current ecological and economical importance.

184 EARLY TOXICITY OF T-2 TOXIN: EFFECTS ON HUMAN MONOCYTE, MACROPHAGE AND DENDRITIC CELL.

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The aim of this work was to study the in vitro effect of T-2 toxin on human monocyte differentiation. Two aspects have been explored: cytotoxicity of T-2 toxin on monocytes, and interaction of T-2 toxin with differentiation process into macrophage and dendritic cells. T-2 toxin previously shown to be myelotoxic for human haematopoietic progenitors has been tested on monocyte-derived from human umbilical cord blood. Results show that T-2 toxin is strongly cytotoxic. The IC 50 is equal to 3.8.10-8 M, 2.10-8 M and 5.10-9 M after 24H, 48H and 72H of incubation respectively. Effects of T-2 toxin on differentiation of monocytes into dendritic cells have been explored using several parameters: phenotypic expression (CD1a, CD14) and endocytic capacity. In the presence of tested trichothecene during differentiation of monocyte into dendritic cell, endocytic activity is maintained, CD1a is down-regulated and CD14 is up-regulated. Effects of T-2 toxin on differentiation of monocytes into macrophage have been explored using five parameters: phenotypic expression (CD71, CD14, CD11a, CD80, CD86, HLA-DR and CD64), endocytic capacity, phagocytosis, burst respiratory activity and TNF-α secretion. The presence of T-2 toxin during differentiation of monocyte into macrophage, induce an up-regulation of CD71 expression, a reduction of capacity of endocytosis, phagocytosis and a strong reduction of burst respiratory activity. Cells exposed to T-2 toxin during differentiation process poorly secret TNF-α. Those results show that T-2 inhibits differentiation of human monocytes into macrophage and dendritic cells. These results could significantly contribute to immunosuppressive properties of this alimentary toxin.

185 LESSONS FROM THE IMMUNOTOXICOLOGY BENCH: A MULTI-PLATFORM APPROACH TO INNATE IMMUNE EVALUATION OF POTENTIAL IMMUNOTOXICITIES.

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Innate immunity is an organism's first line of defense against foreign antigens including microbes, toxicants and malignant cells. Although innate immune responses lack the specificity of antibody or lymphocytic cell-mediated immunity, innate immunity is no less complex, consisting of physical, cellular and chemical components. Thus, investigating multiple components and functions will putatively provide a more accurate measurement of innate immune status. Popular assessments include cytokine evaluation, polymorphonuclear (PMN) phagocytosis and respiratory burst function, and natural killer cell (NK) activity. While PMN function and NK activity methods are abundant in the literature, many are not conducive to multi-faceted, high-throughput evaluations necessary to rigorously test drug effects in pre-clinical species. We have optimized flow cytometric-based phagocytosis and respiratory burst assays using microliter quantities of peripheral blood from rodents, dogs and monkeys for both in vitro and ex vivo assessments. Phagocytosis and respiratory burst functional analyses of both neutrophils and monocytes are independently acquired based on size and granularity within the same sample. NK activity assays for rodents and monkeys have been optimized using two-color flow cytometric analysis, DiO-labeled target cells and Topro® viability dye, eliminating Cr^{51}, while increasing sensitivity. Concurrently, multiplex cytokine profiling has been successfully employed. The cell-based and cytokine analyses are performed in 96-well format allowing ease of multiple experimental parameters including drug concentration, treatment time, stimulation conditions (eg, *E.coli* and phorbal esters for respiratory burst, IL2 and IFN-γ for NK activity) and large population size. This platform has been used to successfully characterize both well-known and novel immunomodulators and is proposed as an efficient and rigorous approach to investigate pharmacologic and/or toxicologic alterations in innate immune function.

186 ARSENITE EXPOSURE DECREASES FUNCTIONAL ACTIVATION RESPONSE AND BACTERICIDAL CAPABILITY OF LPS-STIMULATED J774A.1 MURINE MACROPHAGES.

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Inorganic arsenic (iAs) is an ubiquitous element with immunotoxic effects in different cellular types. Arsenite exposure in monocytes alters differentiation, expression of pro-inflammatory cytokines and the production of nitric oxide ($NO^{\bullet}$), although the molecular mechanisms remain to be defined. The aim of this study was to establish the effect of the *in vitro* exposure to arsenite on $NO^{\bullet}$ and reactive oxygen species (ROS) production, secretion of TNF-α, IL-1β, IL-6, IL-10, ERK1/2 and p38 activation, and the bactericidal capability of functionally stimulated macrophages. We used a murine macrophage cell line (J774A.1) stimulated with 1 μg/ml of LPS, and treated with arsenite (100 nM to 5 μM). Citotoxicity was evaluated by MTT, $NO^{\bullet}$ and ROS were by nitrates and DCFH-DA oxidation; cytokine secretion was evaluated by ELISA, while ERK1/2 and p38 activity were determined using a commercial kit. The bactericidal capability against *Mycobacterium microti* was evaluated *in vitro* in infected J744A.1 cells. Arsenite exposure (0.1 to 1 μM) in stimulated macrophages failed to affect their viability, but decreased $NO^{\bullet}$ and ROS production. Arsenite decreased TNF-α and IL-1β secretion, but increased the secretion of IL-6 and IL-10. In stimulated cells 1μM of arsenite significantly induced the ERK1/2 activity at 30 min, and decreased the p38 activity; exposure to 0.5 μM arsenite decreased the bactericidal capability of the LPS-stimulated macrophages. Overall, our results support that exposure to non cytotoxic concentration of arsenite negatively affect functional activation and the bactericidal capability of the J774A.1 macrophages. These effects may be related with immune depression of cell-mediated protection, a mechanism potentially underlying the development of cancer and intracellular infections associated to iAs exposure. *This work was partially supported by Conacyt-46297.*

187 IMMUNOTOXIBASE: A FREE-ACCESS DATABASE ON THE IMMUNOTOXICITY OF DRUGS AND CHEMICALS.

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There is a growing concern with regards to the potential immunotoxicity of drugs and chemicals. Although a fair amount of data has been published, it is often difficult to identify all sources of information comprehensively, and then to analyze the actual relevance and toxicological signification of the available data adequately. Indeed, the methodology used and the very nature of reported findings may sometimes lead to misleading or inaccurate conclusions on the immunotoxic potential of drugs and chemicals. In an attempt to fill this gap, IMMUNOTOXIBASE (http://www.immunotoxibase.com) is a free-access database that is established to offer a comprehensive coverage of all published data on every medicinal product and chemical whatever its use and source. An extensive description of both non-clinical and clinical data together with detailed information on published data in tabulated format is provided for each molecule individually with a complete reference list. Individual molecules are grouped in families of close chemical structure. The data are searchable by molecule, families, type of endpoints, and species. By providing free access to data whose toxicological relevance has been carefully evaluated, IMMUNOTOXIBASE can improve communication to health professionals and the general public on the immunotoxic effects of a wide variety of drugs and chemicals.

188 EXPOSURE TO CARBON BLACK NANOPARTICLES ACTIVATES ANTIGEN-PRESENTING CELLS *IN VIVO* AND *IN VITRO*.

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Particulate matters including carbon black (CB) nanoparticles can enhance antigen-related inflammation and immunoglobulin production. The present study was designed to determine 1) the effects of different sizes of CB nanoparticles on the antigen-presenting cells (APC) in the lung in vivo, and 2) whether CB nanoparticles affect on maturation/activation and function of dendritic cells (DC) as professional APC in vitro.

In vivo study, mice were exposed to vehicle, 14nm or 56nm CB nanoparticles, ovalbumin (OVA), or OVA+CB nanoparticles intratracheally. The expression of MHC class II, costimulatory molecules (CD80, CD86, CD11c), and DEC205 (DC marker), F4/80 (macrophage marker), and CD19 (B-cell marker) in the lung cells was measured by flow cytometry. In vitro study, bone marrow-derived DC (BMDC) were exposed to 14nm or 56nm CB nanoparticles. The expression of MHC class II, DEC205, CD80, and CD86 was measured by flow cytometry. BMDC function was evaluated by allogeneic mixed lymphocyte reaction (MLR).

14nm, but not 56nm CB nanoparticles, increased the number of the total lung cells. OVA plus 14nm or 56nm nanoparticles increased the total lung cells. The expression of MHC class II and/or costimulatory molecules and the number of APC in the lung were increased by 14nm nanoparticles in the presence or absence of OVA. The increases were more prominent with combination of OVA+14nm nanoparticles. 56nm nanoparticles did not show the significant effects. Either 14nm or 56nm nanoparticles enhanced the expression of MHC class II and/or DEC205, CD80, and CD86 of BMDC. BMDC-stimulated MLR was also enhanced by exposure to CB nanoparticles. The function of BMDC exposed to 14nm nanoparticles was partially and significantly greater than that to 56nm nanoparticles.

CB nanoparticles can induce APC activation, which may be related to their enhancement of allergic responses.

189 SUPPRESSION OF B CELL DIFFERENTIATION BY 2, 3, 7, 8- TETRACHLORODIBENZO-P-DIOXIN.

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The term dioxin is used for members of the polyhalogenated aromatic hydrocarbons that have a common spectrum of responses mediated via binding to a specific high affinity protein. The prototype chemical for this class is 2,3,7,8-tetrachlorodibenzo-para-dioxin (TCDD). Previous studies in our lab have shown that TCDD induced suppression of IgM antibody response via a AhR/Arnt/DRE interaction. Previous studies have demonstrated that CD40 ligand is able to stimulate human B cell differentiation. In this research we wanted to determine the effectiveness of CD40 ligand (CD40L) and lipopolysaccharide (LPS) as stimuli for B cell differentiation. Mouse splenocytes were co-cultured with LPS and purified B cell from the splenocytes were co-cultured with CD40L along with different concentrations of TCDD. The antibody forming cell (AFC) response and anti-IgM ELISA were performed. The AFC response assays concluded that LPS stimulated mouse B cell differentiation which is not suppressed by TCDD. AFC response, and IgM ELISA performed using CD40L cells showed a good induction of the IgM response in both assays. In the two cases TCDD at 30nM suppressed the IgM AFC response. These studies have implicated that CD40L sufficiently stimulated B cell differentiation and TCDD inhibited this stimulation.

190 SUBCELLULAR LOCALIZATION OF THE AMIDE CLASS HERBICIDE 3, 4-DICHLOROPROPIONANILIDE (DCPA) IN T CELLS.

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3,4-dichloropropionanilide (DCPA), or propanil, a post-emergent herbicide used on rice in the United States, is immunotoxic in vivo and in vitro. Although it has been shown that DCPA exerts differential effects on specific immune cell types, the subcellular localization from which DCPA modulates the immune cells has not been identified. In this study, Jurkat T cells were exposed to 100 microM DCPA for one hour after stimulation with phorbol myristate acetate (PMA) and A23187. Following incubation, we used differential detergent fractionation (DDF) coupled to centrifugation to isolate the cytosolic, membrane/organelle, and nuclear fractions of the cells. DDF relies on detergents to sequentially extract proteins from eukaryotic cells. Purity of cell fractions was determined by Coomassie staining and probing for specific subcellular markers using Western blot. DCPA, when present, was removed from each cell fraction using a liquid-liquid extraction method. The extraction product was then analyzed for the presence of DCPA using electrospray ionization-ion trap tandem mass spectrometry (ESI-MS/MS), in the negative ion mode. The presence of both DCPA's precursor ion and product ion was considered to be positive detection. The analyses demonstrate that DCPA, a lipophilic compound, localizes primarily in the cytosol. Very low levels of DCPA were detected in membrane/organelle fractions, while DCPA was absent from the nuclear fractions. These results indicate that DCPA is able to cross the plasma membrane and likely initiates its immunomodulatory effects from inside the cell. *MLH and CJP contributed equally to this study.

191 EVALUATION OF VIRAL REACTIVATION BY MURINE IFNβ AND AN α4 INTEGRIN MONOCLONAL ANTIBODY IN A MCMV HOST RESISTANCE MODEL.

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Immunosuppression may result in reactivation of various latent viruses (e.g. JCV, CMV), which can lead to serious and/or life threatening disease. Reactivation of JCV may result in progressive multifocal leukoencephalopathy (PML), a rare demyelinating disease of the CNS. PML has been seen in association with a number of small molecule and biologic therapeutics. However no animal model for PML exists, making it difficult to evaluate factors contributing to reactivation. Rodent models of CMV may provide an alternative system to investigate treatment-related viral reactivation. This study evaluated murine CMV reactivation in Jh B-cell-deficient mice treated with PS/2 (rat anti-mouse α4 integrin monoclonal antibody), murine IFN-β, or combination treatment (PS/2 + murine IFN-β). Mice were infected with MCMV and primary infection was confirmed on Day 28 by measurement of infectious virus in salivary glands. Latency was established at Week 12 by the absence of infectious virus. At latency, mice were treated with: 1) a cocktail of depleting antibodies (to CD4, CD8 and NK1.1), 2) IgG2b isotype control, 3) PS/2 (5 mg/kg), 4) IFN-β (10,000 IU), and 5) PS/2 + IFN-β (same doses). Mice were treated for 14 days and necropsied 9 days after the last treatment. Treatment of mice with cell-depletion cocktail resulted in reactivation of latent virus in 18 out of 18 mice, whereas treatment with IgG2b isotype control did not result in reactivation (0/18). By contrast, treatment with PS/2, IFNβ or the combination resulted in reactivation in 1/18, 3/18 and 2/17 mice, respectively. These results demonstrate the MCMV system is very sensitive for identifying low level viral reactivation events that may result from treatment with immunomodulatory compounds. However, the relationship of these results to real world outcomes is unclear, as IFNβ treatment in humans is not associated with viral reactivation.

192 AUGMENTATION OF INFLAMMATORY MEDIATORS IN AQUEOUS HUMOR AND DIFFERENTIAL REMODELING OF THE CORNEAL STROMA IN RESPONSE TO SULFUR MUSTARD EXPOSURE.

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Acute ocular injury resulting from mustard agent exposure resembles an inflammatory response involving the infiltration of lymphocytes and the production of inflammatory mediators. The purpose of this study was to investigate the profile of cytokines, chemokines, and matrix metalloproteinases (MMP) in the aqueous humor (AH) of sulfur mustard exposed-eyes in rabbits, and to correlate histological changes in chronically-damaged cornea with a putative pro-inflammatory response. Interleukin (IL)-1β, tumor necrosis factor (TNF)α, IL-6, IL-8, MMP-2, and MMP-9 were found to be upregulated in the AH of animals exposed to sulfur mustard. Transition electron microscopy of exposed corneas demonstrated necrosis in surface epithelial cells, separation of the epithelium from the stroma, disruption of Bowman's layer, separation of lamellae and disorganization of collagen fiber. These architectural changes are consistent with direct tissue injury from mustard treatment as well as structural disruption due to the activity of MMPs. Together, these data suggest that ocular sulfur mustard exposure activates inflammatory mediators which may contribute to the activation of the MMPs in AH. Furthermore, this inflammatory response may play a role in the chronic corneal injury resulting from sulfur mustard exposure. Further investigation into the signaling pathway(s) and the contribution(s) of each of the mediators may determine therapeutic points of intervention for acute and chronic ocular sulfur mustard exposure.

193 EFFECT OF TARGETED ANTI-INFLAMMATORY AGENTS IN A RAT STREPTOCOCCAL HOST RESISTANCE MODEL: IL-1RA, ANTI-TNF, OR COMBINED IL-1RA AND ANTI-TNF.

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Streptococcus pneumoniae is a Gram positive coccus that is a human pathogen and an important etiological agent in diseases of the upper respiratory tract including bacterial pneumonia, sinusitis and otitis media. Bacterial clearance in the S. pneumoniae host resistance model is mediated by innate immune mechanisms. The present study used S. pneumoniae as a pulmonary bacterial host resistance model in Lewis rats to evaluate the targeted anti-inflammatory agents: Anti-TNF (fusion protein coupled with the Fc portion of human IgG1 which binds to the TNF receptor, also known as etanercept/enbrel®); IL-1Ra (recombinant human interleukin-1 receptor antagonist, also known as anakinra/kineret®); and a combination of anti-TNF and IL-1Ra. IL-1Ra was administered by subcutaneous continuous infusion of approximately 0.7 mg/hr (17.9 mg/day) via an osmotic minipump, starting 3 days prior to infection. Anti-TNF (10 mg/kg) was injected subcutaneously 48 hours and 2 hours prior to infection. Dexamethasone (30 mg/kg), administered by oral gavage 1 hour prior to infection, was used as a positive control. Rats were infected intranasally with S. pneumoniae and sacrificed 6 or 24 hours post-infection. Terminal body, spleen and thymus weights, bacterial clearance and measurement of IL-6, TNFα and IL-1β in the lung, and blood CBCD and various cytokines were evaluated. Following infection, IL-1β, IL-6, and TNFα in the lung and blood neutrophil counts were increased over pre-infection levels. Absolute neutrophils were slightly decreased in rats treated with anti-TNF or dexamethasone. There were no significant changes in body, spleen, or thymus weights in any of the rats treated with IL-1Ra, anti-TNF, or the combination treatment. Treatment with dexamethasone decreased thymus and spleen weights. Bacterial clearance was impaired following treatment with anti-TNF and dexamethasone, but not by treatment with IL-1Ra or the combination treatment.

194 *IN VITRO* IMMUNOTOXIC EFFECTS OF MERCURY DEPEND ON ACTIVATION OF HUMAN PBMCS.

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Immunotoxicants, such as mercury (Hg), may interact with infections, antigens, or other activators of the immune system. These interactions, which include immunosuppresion (in the case of malaria) and autoimmunity (in the case of coxsackievirus B3 or allograft), occur under conditions of Hg exposure that do not induce overt toxicity or loss of immune cells. We therefore hypothesize that the effects of low dose Hg in cell systems will depend upon activation of the immune response. To test this hypothesis, we have compared the in vitro effects of inorganic Hg on human peripheral blood mononuclear cells (PBMCs) from healthy volunteers in the presence and absence of LPS (lipopolysaccharide) stimulation. We investigated responses to Hg in PBMCs from 20 volunteers who donated blood six times over a period of 6-18 months. PBMCs were exposed to increasing concentrations of $HgCl_2$ from 10 to 200 nM. These exposures, which roughly correspond to a whole blood concentration at the upper range of occupationally exposed or fish eating populations, are noncytotoxic. Cytokines were measured by bead-based multiplex suspension assays (Bio-Rad). As expected, treatment with LPS alone caused large increases in the production of the pro-inflammatory cytokines TNFα, IL-1β, and IFNγ, a small but significant increase in IL-4 production, and no change in IL-17 production, compared to non-stimulated controls. In the presence of LPS stimulation, Hg caused further dose-dependent increases in pro-inflammatory cytokine production (TNFα, IL-1β, and IFNγ) in PBMCs from both males and females. No changes were observed in IL-4 or IL-17 levels in response to Hg treatment. Moreover, no changes were observed for any cytokine at any concentration of Hg in the absence of LPS. This research indicates that the immunotoxic properties of Hg are elicited in the presence of immune activation and provides further evidence that Hg is an immunotoxic substance of concern to human health.

195 DEVELOPMENT OF TOLERANCE TO Δ9-THC-INDUCED SUPPRESSION OF *IN-VIVO* AND *IN-VITRO* HUMORAL IMMUNE RESPONSES.

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In B6C3F1 mice, THC administered orally results in marked suppression of T-cell dependent humoral immune responses to sheep erythrocytes (sRBC) as measured both *in-vivo* with the IgM Antibody Forming Cell (AFC) assay [Plaque assay] and following *in-vivo* exposure and *in-vitro* sensitization; a modified Mishell-Dutton (MD) assay. The objectives of these studies were to determine whether tolerance develops to these effects of THC utilizing doses of 5, 15.8, 50, and 158 mg/kg THC and dosing durations of 5, 28 and 45 days; and, to ascertain the cell type(s) responsible for adversely affecting the humoral immune response. In the Plaque assay after 5 days of exposure mice treated with 50 or 158 mg/kg THC had significantly suppressed AFC responses compared to vehicle controls. When the exposure period was lengthened to 28 days, the AFC response was only significantly suppressed at the highest dose level. Furthermore, after 45 days of exposure the responses of animals from all dose levels were nearly identical to vehicle treated mice, indicating tolerance had developed. The *in-vitro* MD response was significantly suppressed at all dose levels at or above 15.8 mg/kg after 5 days of THC exposure; the 45 day study is ongoing. To identify which splenic immune cell type(s) mediate the observed immunosuppression, spleen cell suspensions from mice treated for 5 days with either vehicle or 158 mg/kg THC were separated into plastic adherent and non-adherent populations and reconstituted prior to sensitization *in-vitro*. These studies demonstrate that non-adherent cells (T- and B-cells) and adherent cells (antigen presenting cells) from THC treated mice contributed to the suppressed MD responses when compared to respective controls. These results demonstrate that tolerance develops to Δ9-THC induced humoral immunosuppression with repeated administration following *in-vivo* sensitization. Furthermore, the study highlights the utility of the MD assay for identifying adversely effected cell populations and potential mechanisms of action. Supported by NIDA T32DA007027, F31DA022849.

196 A CONSTITUTIVELY ACTIVE AHR EXPRESSED IN T CELLS INCREASES PERCENTAGE OF $CD25^+CD4^+$ T CELLS BUT DOES NOT SUPPRESS ANTIBODY PRODUCTION UPON OVA-IMMUNIZATION OF MICE.

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Exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) activates the transcription factor aryl hydrocarbon receptor (AhR) and triggers diverse biological reactions, including induction of thymus atrophy, generation of $CD25^+CD4^+$ T cells with functional characteristics of regulatory T (Treg) cells, and suppression of Th2 cytokine production and antigen-specific antibody production. To investigate the exact role of the AhR in T cells we previously generated transgenic (Tg) mice that express a constitutively-active mutant of AhR (CA-AhR) specifically in T-lineage cells. In the present study we examined the primary and secondary immune reactions of the Tg mice immunized with ovalbumin (OVA). The increases in spleen T cell and B cell numbers upon primary immunization with OVA were significantly suppressed in the Tg mice comparing to the wild-type littermates. The percentages of $CD25^+CD4^+$ cells among the thymocytes and among the spleen $CD4^+$ T cells were higher in the Tg mice than in the wild-type mice. However, Th2 cytokine (IL-4 and IL-5) production by splenocytes and the amount of anti-OVA IgM in the

serum did not significantly differ between the Tg mice and wild-type mice. IFN-γ production by the splenocytes was significantly augmented in the Tg mice comparing to the wild-type mice. After the secondary immunization, the levels of Th2 cytokine production and anti-OVA IgG1 were also not different between the Tg mice and wild-type mice. These results indicate that AhR activation in the T cells alone suppresses the immunization-induced increase in T cells and B cells and increases the percentages of $CD25^+CD4^+$ T cells. On the other hand, they also suggest that AhR activation in other cells, such as antigen-presenting cells and B cells, is involved in the TCDD-induced suppression of Th2-cytokine production and antibody production.

197 ENHANCED HOST RESISTANCE TO LISTERIA MONOCYTOGENES (LM) FOLLOWING EXPOSURE TO THE POTENT IMMUNOSUPPRESSIVE COMPOUNDS BENZO(A)PYRENE AND DIBENZ(A, H)ANTHRACENE IN FEMALE B6C3F1 MICE.

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Benzo(a)pyrene (BAP) and Dibenz(a,h)anthracene (DBA) are polycyclic aromatic hydrocarbons (PAH),which are formed by incomplete combustion of carbon-containing fuels,fires, and tobacco-smoke.The immunosuppression produced by BAP has been extensively studied.Previously, our laboratory has reported that humoral immunity is primarily suppressed. After 14 days of daily subcutaneous exposure at 5, 20, or 40 mg/kg, BAP exposure produced decreased host resistance (HR) in virus, parasite, and tumor models. Similarly, HR to the bacteria, Streptococcus pneumonia (SP), was dose dependently decreased.Paradoxically, in the LM bacteria model BAP produced a dose related enhancement of host resistance, resulting in a statistically significant decreased moribundity in BAP treated animals when compared to vehicle controls.DBA is a more potent immunosuppressive congener of BAP, producing similar effects on humoral immunity but at lower doses.In addition to adversely effecting innate and cell-mediated immunity, DBA also decreased HR to SP when evaluated by spleen and liver colony counts.Consistent with BAP results, animals treated with DBA were protected from LM and had enhanced HR when evaluated by spleen and liver colony counts. Our initial hypothesis was that treatment with BAP or DBA would result in an increase in IFNg, which activates macrophages, resulting in killing of LM intracellular pathogens. This was supported by a dose dependent increase in IFNg mRNA obtained from splenocytes of DBA treated animals, following stimulation with LPS in culture. However, supernatant of splenocytes from animals treated with DBA cultured with LPS for 24 or 48 hr had decreased levels of IFNg compared to the vehicle controls. Current studies are focused on binding of PAHs to AHR and how this event leads to enhanced HR to LM. Our data demonstrate that PAHs which do not bind AHR are susceptible to LM, consistent with the decreased HR to LM in AHR null mice. Supported by the NIEHS Contract ES 05454.

198 THE EFFECTS OF PERFLUOROOCTANE SULFONATE EXPOSURE ON INFLAMMATION IN B6C3F1 MICE.

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Perfluorooctane sulfonate (PFOS) is one of several perfluorinated compounds (PFCs), an emerging class of environmental contaminants. PFCs are globally distributed, having been identified in environmental and wildlife samples worldwide, with PFOS constituting the highest concentrations. Few studies to date, however, have assessed the immunotoxicity of PFOS or specifically its effects on inflammation. This study utilized a standard 28-day oral exposure according to OPPTS immunotoxicity test guidelines to assess the effects of PFOS on inflammatory cytokine levels in plasma(TNF-alpha and IL-6) and peritoneal lavage fluid (TNF-alpha and IL-6), intracellular splenic cytokine levels (IL-1, IL-6, IL-10, and TNF-alpha), cytokine production by peritoneal macrophages (TNF-alpha and IL-6) and percent splenic macrophages (F4/80+ cells). Adult female B6C3F1 mice were dosed daily by oral gavage with 0, 0.036, 0.107, or 10.714 mg/kg/day to yield a targeted Total Administered Dose (TAD) over the 28 days of 0, 1, 3, or 300 mg PFOS/kg. PFOS exposure significantly reduced plasma TNF-alpha levels at 1 mg/kg but not at 3 or 300 mg/kg. Peritoneal lavage fluid had significantly lower IL-6 levels following exposure to 300 mg/kg only while TNF-alpha was not affected. Splenocyte intracellular levels of IL-6, Il-1, IL-10 and TNF-alpha were not altered compared to control. Ex vivo TNF-alpha production by peritoneal macrophages was significantly higher in mice dosed with 300 mg/kg whereas IL-6 secretion was not altered. Finally, percent splenic macrophages was significantly increased only at the 3 mg/kg treatment. These data suggest that exposure to PFOS modulates inflammatory responses however; further research is needed to determine the mode/mechanism of action.

199 HPLC/DAD STUDY ON COVALENT BINDINGS OF SENSITIZING CHEMICAL SUBSTANCES TO SERUM ALBUMIN.

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The covalent bindings of sensitizing chemical substances to serum albumin were analyzed by high-performance liquid chromatography with photo-diode array detector (HPLC/DAD). 2,5-Heanedione, glutaraldehyde, phthalic anhydrate, phthalic acid, bisphenol A diglycil ether (BADGE), bisphenol A, diaminodiphenyl ether, and toluene 2,4-diisocyanate (TDI) were subjected in this study. The chemicals were directly incubated with albumin and/or were once dissolved in DMSO and incubated with albumin in phosphate buffer at room temperature. The chemicals, albumin and mixtures were applied to HPLC/DAD. The control (albumin without chemicals) showed a single peak at 32 min retention time with two peaks of absorbance at 200 nm and at 280 nm. The retention time of chemicals and albumin were different. And albumin treated with chemicals showed retention time delay from the original albumin, and have different peaks of absorbance. The albumin treated with some chemicals showed more than two peaks of different retention time. It means that chemicals form more than two different structures of chemico-albumin complexes. These sensitizing chemicals are too small molecule size to be an epitope by themselves. These designated sensitizing chemicals were demonstrated to change the structure of the albumin and elicit sensitization. Other considerations include which peaks of the chemico-albumin complexes show imunogenesity. Separated sample of the chemico-albumin complexes were analyzed the imunogenesity by the patients serum who suffered from allergies to environmental chemicals.

200 AN ELECTROPHORETIC STUDY ON COVALENT BINDINGS OF SENSITIZING CHEMICAL SUBSTANCES TO ALBUMIN.

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The covalent bindings of sensitizing chemical substances to human serum albumin were analyzed by sodium dodecyl sulfate-polyacryamide gel electrophoresis (SDS-PAGE) and isoelectric focusing. 2,5-Hexanedione, glutaradehyde, phthalic anhyderate, phthalic acid, bisphenol A diglycidyl ether (BADGE), bisphenol A, diaminodiphenyl ether, toluene 2,4-diisocyanate (TDI) were subjected in this study. The chemicals were dissolved in DMSO and incubated with human serum albumin (HSA) in phosphate buffer at room temperature. The mixture were applied to SDS-PAGE and stained by Coomassie brilliant blue. Control (HSA without chemicals) showed a big band at 66 kDa. HSA treated with Glutaradehyde, phthalic anhyderate showed different bands from the original HSA band. HSA treated with BADGE and TDI showed a broad band at the molecular weight of 66 kDa. On the other hand, the electrophoretic patterns of 2,5-hexanedione, phthalic acid, bisphenol A and diaminodiphenyl ether were same as that of control. Same tendencies were observed in the analysis by isoelectric focusing. Glutaradehyde, phthalic anhyderate, BADGE and TDI are designated as sensitizers by Japan Society for Occupational Health, American Conference of Governmental Industrial Hygienists, Deutschen Forschungsgemeinschaft and European Union. These designated sensitizing chemicals were demonstrated to change the tertiary structure of HSA and elicit sensitization. HSA treated with the chemicals was analyzed by western blotting with patients who suffered from allergies to environmental chemicals. A patient with bronchial asthma by TDI had a specific IgE to HSA treated with TDI.

201 EVALUATION OF THE EFFECTS OF NYLABONES® ON RAT SUBCHRONIC TOXICITY STUDY ENDPOINTS.

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Environmental factors can confound responses in toxicity studies. There are few reported studies of the impact of common environmental enrichment devices on these responses. The objective of this study was to evaluate the effects of Nylabone® exposure on standard subchronic toxicity study endpoints in rats (in-life, neurobehavioral, clinical and anatomic pathology parameters). In each of two 90-day feeding studies, satellite groups (NB) of 10 male and 10 female rats were housed in the same room and evaluated for the same endpoints as study rats (SC). NB rats were treated the same as SC rats except each was provided with a Nylabone®. Data from NB rats were compared to those from SC rats. No notable differences were observed between NB and SC groups for most endpoints. Observed differences included slightly higher body weight gain, food consumption, and food efficiency in NB females, but not in NB males, in both studies. Unilateral trigeminal nerve degeneration was seen in many NB rats in the first study, but was

not reproduced in the second study. These results support that the use of Nylabones® for environmental enrichment is unlikely to confound results in general toxicity studies.

202 OCULAR PHOTOTOXICITY: ROLE OF PIGMENTATION AND OBSERVED PHOTOTOXICITY INDUCED BY 8-METHOXYPSORALEN.

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Evaluation of ocular phototoxicity often includes a comparator article group to ensure that the experimental system is capable of detecting the endpoint of concern. Because of the "reservoir hypothesis" regarding pigment-bound drugs, test systems with ocular melanin are now routinely required. Albino animals play an important role in providing a test in the absence of melanin pigment. Phototoxicology testing involves exposure to solar-simulated light (SSL, ultraviolet radiation 290-400 nm; visible light, 400-700 nm). For the current study, Sprague-Dawley (SD) and Long-Evans (LE) rats were orally-administered (by gavage) the phototoxic comparator 8-methoxypsoralen (8-MOP). The eyes of the rats were then exposed once to SSL. Ophthalmologic evaluations (indirect and slit lamp with photography) were performed 1, 3, 7 and 14 days after SSL exposure. SD and LE rats had corneal epithelial edema, metaplasia and erosions, secondary uveitis with vascular congestion and chronic interstitial keratitis. Histopathologically, in SD rats, cataractous changes occurred along with corneal findings consistent with the ocular findings and retinal degeneration affecting the photoreceptor layer, outer nuclear layer, outer plexiform layer, inner nuclear layer and ganglion layer.. LE rats exhibited corneal changes but had no evidence of cataracts or retinal changes. Focal retinopathy, characterized by a minimal focal area of loss of the outer nuclear area and photoreceptor area occurred in a small number of both SD and LE rats administered vehicle or 8-MOP but was not considered indicative of phototoxicity. These results indicate that the pigmentation status of the Test System can affect the phototoxic response, in this case associated with reduced damage. A different comparator drug would be required for testing whether ocular melanin could function as a reservoir for the release of phototoxic substances.

203 CHRONIC INTERMITTENT EXPOSURE TO SOLAR-SIMULATED ULTRAVIOLET AND VISIBLE RADIATION INDUCES OCULAR CHANGES IN ALBINO BUT NOT PIGMENTED MICE.

D. B. Learn[1], J. D. Wilson[2], C. P. Sambuco[1], P. D. Forbes[1], S. Bistner[2], K. Frazier[2], W. R. Brown[3] and A. M. Hoberman[1]. [1]*Center for Photobiology, Charles River Laboratories Preclinical Services, Horsham, PA,* [2]*GlaxoSmithKline, King of Prussia, PA and* [3]*Research Pathology Services, New Britain, PA.*

Evaluating the ocular photosafety of pharmaceuticals can involve complex study designs, unfamiliar technologies and multiple variables. Acute versus chronic exposure, continuous versus discontinuous exposure, pigmentation in the eye structures, dark repair of light-induced damage and melanin binding all may affect toxicity and be must be accounted for to ensure accurate risk assessment. Female albino CD-1 and pigmented C3B6F1 mice were orally (by gavage) administered a simple vehicle formulation daily for twelve consecutive weeks. Five days per week for 12 weeks, the free-moving mice were exposed to 0, 120, 240, 360 or 720 Robertson Berger Units (RBU)/day of xenon arc solar-simulated light (SSL), including ultraviolet radiation (UVR; 290-400 nm) and visible light (400-700 nm). Evaluation of the eyes of all mice using indirect ophthalmoscopy and slit lamp observations during weeks 4, 6, 8, 10 and 12 found no adverse changes. Histopathologic evaluation revealed no lesions in the eyes of C3B6F1 mice at SSL doses as high as 360 RBU/day. CD-1 mice exposed to 360 RBU/day exhibited lenticular degeneration (cataracts) with minimal to mild fiber swelling, nuclear disorganization and/or nuclear debris within the area of the nuclear bow, rare morgagnian globules and occasional presence of hyperplasia, necrosis or fibrous change of the lens epithelial layer in 4 of 10 mice (8 eyes). Retinal degeneration occurred in 1 of 10 mice (1 eye). These findings demonstrate that chronic discontinuous light exposure, even at this elevated intensity and including UVR, induced only a moderate incidence of eye lesions in albino mice, these lesions were only revealed by histopathological evaluation and no lesions were induced in pigmented mice.

204 DEVELOPING *IN VIVO* CEES-INDUCED SKIN TOXICITY MOUSE MODELS.

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Sulfur mustard (HD) is an alkylating cytotoxic vesicant which inflicts severe chemical burns and debilitating skin blistering. Its destructive properties and lack of antidote has made it an effective chemical warfare weapon since World War I and its potential further use by terrorists remains a significant threat. We used a less toxic analog of HD, 2-chloroethyl ethyl sulfide (CEES) to develop a CEES-induced skin toxicity model using SKH-1 female hairless mice. The goal of our study was to identify in vivo inflammatory biomarkers of CEES-induced dorsal skin injury in dose (0.5-2 mg/kg) and time-response (3 h to 168 h) experiments. Topical CEES treatment at high doses caused significant erythema and a dose-dependent increase in skin bi-fold thickness and edema. Histopathological evaluation of CEES-exposed hematoxylin and eosin stained integument revealed blister formation, some desquamation of epidermis and a pronounced dose and time-dependent increase in epidermal thickness and number of pyknotic basal keratinocytes. This analysis was confirmed by immunohistochemical evaluation where terminal deoxynucleotidyl transferase-mediated dUTP nick end labeled–positive cell number increased in a dose-dependent manner demonstrating direct DNA damaging effect of CEES. Proliferative cell nuclear antigen staining showed that CEES also induces basal epidermal cell proliferation. The myeloperoxidase activity in inflamed skin peaked at 48 h after CEES exposure coinciding with neutrophil infiltration. The number of macrophages also increased after 24 h of CEES exposure. We have developed these primary quantifiable inflammatory biomarkers using fast and reliable methods which are currently being used to carry out efficacy studies with various agents against CEES-induced skin toxicity. This model would further help in screening and developing new countermeasures to limit the morbidity and mortality caused by HD.

205 DEVELOPMENT OF AN ANIMAL MODEL OF ABRADED SKIN.

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For pharmaceutical products intended for dermal application, the minipig is an ideal animal model due to the closeness of the skin of this species to human skin. Under normal conditions, the skin constitutes a relatively profound barrier to the external surroundings. However, in some human skin diseases, superficial abrasions and sores develop, potentially increasing systemic absorption of substances intended for topical skin use. This issue is not covered when using healthy animals with intact skin in regulatory toxicity testing of dermal products. For such studies, development of a model for repeated administrations on abraded skin is becoming relevant and we used the Göttingen minipig in regulatory toxicity testing of dermal products, to address this issue. The model combines an initial wound healing phase with subsequent subcutaneous dosing, the latter commencing at complete wound closure. The model has been accepted by the regulatory authorities. Full-thickness wounds are used in the study design as these, in contrast to superficial abrasions, can be inflicted in a very precise and reproducible way. Furthermore, whereas superficial abrasions tend to heal very quickly, the healing process of full-thickness wounds is protracted, enabling time for evaluation of local effects on the wound healing process. It is not considered ethical to wound the animals repeatedly, therefore the initial wound healing phase was combined with a phase of subcutaneous dosing, starting when complete re-epithelialisation of the wounds occurs. This meets requirements for longer duration non-clinical studies and maximises systemic exposure of the test compound. All routine guideline requirements for evaluation of systemic toxicity in non-rodent species are integrated into the study design. We conclude that a combined wound healing and subcutaneous study in minipigs constitutes a valid method for simulation of test compound administration on abraded skin, allowing maximal systemic exposure of the test compound, and evaluation of local effects on the wound healing process.

206 TEMPORAL CHANGES IN MITOCHONDRIAL STRUCTURE, FUNCTION, AND MUTATIONS FOLLOWING *IN UTERO* EXPOSURE OF FEMALE CD-1 MICE TO AZT, 3TC, OR AZT/3TC.

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Treatment with nucleoside analogue reverse transcriptase inhibitors (NRTIs) including zidovudine (AZT) is highly effective in inhibiting viral replication in HIV-infected patients and reducing perinatal transmission of the virus from mother to child; however, emerging evidence suggests that AZT-based therapy increases risk for mitochondrial disease and cardiotoxicity. The objective of this study was to determine the relative impact of NRTIs on mitochondrial structure and function in hearts of mice exposed transplacentally. Pregnant CD-1 mice were treated with AZT, 3TC or AZT/3TC during the last 7 days of gestation. Following echocardiography at 13 and 26 weeks of age, female pups were necropsied and their hearts were sectioned for examination by light and ultrastructural microscopy, measurement of OXPHOS enzyme activity, and measurement of mitochondrial DNA

(mtDNA) depletion and mutations. Echocardiographic analyses revealed a progressive decrease in left ventricular wall thickness that, at 26 weeks of age, was statistically different from controls in AZT-exposed (p=0.021), 3TC-exposed (p=0.006) and AZT/3TC-exposed (p<0.001) mice. Furthermore, left ventricular wall measurements in the AZT/3TC exposed group were significantly less than those in the AZT-alone group (p=0.013) and in the 3TC-alone group (p=0.009). Within each NRTI treatment group, ultrastructural alterations were progressive and distinctive. OXPHOS enzyme activities in complex IV were increased above control values in all exposure groups, with the increases being more pronounced at 26 weeks of age. Assessments of possible treatment-induced mtDNA depletion and mutations, in the 22 mitochondrial tRNA genes, are in progress. The preliminary findings of progressive changes in various biomarkers suggest that this experimental system could be used as a model for investigating chemoprevention of NRTI-induced cardiotoxicity.

207 OZONE EXPOSURE DYSREGULATES KEY ENDOTHELIAL TRANSCRIPTS IN THE APO E (-/-) MOUSE.

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Transcriptome-levele effects of ozone exposure at 300 parts per billion were examined in the endothelium of hypercholesterolemic mice by means of microarray techniques.
Methods: Tie2-GFP mice expressing green fluorescent protein regulated by the endothelial-specific promoter Tie2 were crossed with apolipoprotein E (-/-). Tie2-GFP/Apo E (-/-) mice, 8-20 weeks old were exposed to 300 PPB of freshly generated ozone 4h or 72 h. Aortae (n=4) were dissected from the valve to the iliac bifurcation and rapidly processed by proteolytic digestion followed by fluorescent activated cell sorting to yield >100,000 endothelial cells of >95% purity. Age-matched animals of the same genotype exposed to filtered air alone served as controls. 20 ng of RNA representing >10,000 endothelial cells was subjected to amplification, fluorescent labeling and hybridization to microarrays arrays created from the Operon V3 long oligo set. Analysis was performed with Genepix and Acuity software.
Results and Conclusion: Within the endothelium of Apo E- null and wild-type mice, a set of more than 30 transcripts were dysregulated by > 1.5 fold. Several known or potential effectors of atherosclerosis were differentially dysregulated within Apo E-null vs. wild-type mice following 72 h of continuous exposure to ozone at 300 PPB. Transcripts undergoing enhanced up-regulation in the Apo (-/-) mice at 72 h included carboxyl ester lipase (cel), cxcl2, elastase 3b, trypsin II, kallikrein-related peptidases 5b and 8b, and others. Transcripts undergoing enhanced down-regulation in the Apo E (-/-) mice included fos, mkp-1/dusp1, pai-2, fas and others. Cel, with a known role in cholesterol accumulation, may contribute to enhanced atherosclerosis potentially occurring within the Apo E(-/-) mouse exposed to ozone. By uncovering and confirming endothelial transcripts dysregulated due to a single component of air pollution (ozone at environmentally relevant levels) within a hypercholesterolemic strain, we ultimately hope to reveal the mechanisms underlying pollution-related cardiovascular disease.

208 CONTRIBUTION OF LOCUS COERULEUS DEGENERATION TO THE PARKINSONIAN SYMPTOMS IN VMAT2 DEFICIENT MICE.

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Previously our laboratory has shown that mice with genetic reduction of the vesicular monoamine transporter (VMAT2 LO) display progressive loss of striatal dopamine, L-DOPA responsive behavioral deficits, synuclein aggregation, and nigral dopamine cell loss (J. Neurosci. 27(30):8138-8148). VMAT2 normally acts to sequester catecholamines protecting against neuronal damage; however, when VMAT2 expression is reduced, cytosolic catecholamine levels are increased potentially causing oxidation leading to cellular damage. Here, we report that in addition to the age-dependent dopaminergic degeneration seen in the VMAT2 LO mice, parallel degeneration is seen in the noradrenergic system. Degeneration of the locus coeruleus was observed in aged VMAT2 LO mice. Given our recent finding that norepinephrine deficiency leads to behavioral deficits reminiscent of Parkinson's disease (PNAS 104(34):13804-13809), we hypothesized that the damage to the noradrenergic region and other monoamine systems may produce some of the non-dopamine mediated symptoms of Parkinson's disease. This study investigates Parkinsonian symptoms in aged VMAT2 LO mice at 2 different time points: 6 and 12 months. VMAT2 LO mice displayed decreased novelty-induced locomotor activity compared to wild type animals in both age groups and decreased circadian locomotor activity at 6 months of age. Moreover, at 6 months of age, VMAT2 LO mice have a shorter latency to behavioral signs of sleep than wild type animals. No differences in anxiety-like behavior were observed, as tested by the elevated plus maze, between the VMAT2 wild type and LO animals. These data suggest that these mice may exhibit widespread degeneration that reproduces many of the features of Parkinson's disease. Supported by U54 ES-012068 and the American Parkinson's Disease Association.

209 IMMUNOCYTOCHEMICAL AND BEHAVIORAL EVIDENCES OF THE NEUROPROTECTIVE EFFECTS OF MELATONIN IN A PARKINSON'S DISEASE MODEL.

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Several lines of evidence suggest that reactive oxygen species play a pivotal role in the pathogenesis of Parkinson's disease. Melatonin, a neurohormone produced by the pineal gland, acts as an antioxidant and a free radical scavenger in vivo and in vitro. Levodopa is the most common drug for the treatment of Parkinson=s disease because of its clinical efficacy. However, chronic L-dopa treatment is associated with the development of adverse reactions in the majority of patients. Thus we conducted this study to determine if Melatonin reverses the alterations induced by 6-OHDA and compare the results with L-dopa treatment. 24 Wistar rats were trained in the beam walking test before the lesion, then the animals were stereotactically injected with either 6-OHDA (n=18), or sham lesioned with vehicle (n=6). Two days after the lesion rotational behavior was tested and 2 days later rats were treated with melatonin (n=6) or L-dopa (n=6) orally for a month and 6 lesioned rats without treatment were kept for the same time. At 7, 14, 21 and 28 days after the lesion motor behavior was evaluated. Rats were sacrificed, and ipsi and contralateral substantia nigra were processed for tyroxine hydroxylase (TH) immunocytochemistry. The lesioned rats showed poor performance in the beam walking task at all evaluated times, in contrast either L-dopa or melatonin treated rats exhibited better motor performance, however after 28 days the L-dopa treated rats deteriorated their motor ability; moreover, the L-dopa group shows less number of TH-positive cells than melatonin treated group. Our results show that melatonin prevents in part the dopaminergic cell loss and induces better motor performance, and although L-dopa at the beginning of the treatment seems to work, after 28 days the rats manifested evident motor alterations. Thus, we conclude that the treatment with some antioxidant like melatonin may prove to be of some help in ameliorating the oxidative stress produced by the dopamine depletion.
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210 OZONE DYSREGULATES CARDIAC AND PULMONARY TRANSCRIPTS IN THE APO E (-/-) MOUSE.

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Oxidant air pollution exacerbates respiratory and cardiovascular disease. We examined the effect of acute (4 h) and chronic (72 h) ozone on gene expression in vivo, utilizing hypercholesterolemic transgenic mice. Changes in the abundance of transcripts within total heart and lung were then examined by microarray techniques.
Methods: Tie2-GFP mice expressing green fluorescent protein regulated by the endothelial-specific promoter Tie2 were crossed with apolipoprotein E knockout mice to generate Tie2-GFP/Apo E null mice. Tie2-GFP and Tie2-GFP/Apo E mice (unrestrained) were exposed to ozone at 300 PPB and hearts and lungs rapidly dissected 24 and 72 h following the commencement of acute and chronic exposures. Changes in the abundance of cardiac and pulmonary transcripts were then examined comprehensively by microarray techniques. Pooled RNA was labeled by conversion to cDNA labeled with fluorophore and hybridized to microarrays containing the Operon V3 long oligo set.
Results and Conclusion: Within the hearts and lungs of Apo E- null and wild-type mice, a set of more than 30 transcripts were dysregulated by > 2 fold. By uncovering and confirming cardiac and pulmonary transcripts dysregulated due to air pollution (and its components) within a hypercholesterolemic strain, we ultimately hope to reveal the mechanisms underlying pollution-related cardiovascular disease.

211 RESPIRATPORY SAFETY PHARMACOLOGY: RELEVANT PARAMETERS.

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Current regulatory guidelines (S7A) require that respiratory safety pharmacology be evaluated using parameters such as respiratory rate, tidal volume and hemoglobin oxygen saturation. Recent results suggest that hemoglobin oxygen saturation has a

relatively poor sensitivity to detect adverse effects (Authier et al. in press). Ideally, respiratory safety pharmacology would include measurement of airway resistance which requires the use of surgically implanted pleural probes with telemetry transmitters or the use of a double chamber plethysmograph which requires a very high acquisition rate. A significant number of respiratory safety pharmacology studies are conducted with head-out plethysmographs to obtain ventilation parameters. Our hypothesis was that a number of respiratory parameters can be calculated from the airflow signal and would offer a more sensitive evaluation of respiratory safety than the one mentioned in S7A while using the head-out configuration. Five groups of 8 male Sprague-Dawley received Saline (2 mL/kg), Methacholine (28 and 136 μg/kg), Albuterol (0.4 mg/kg) and Remifentanil (28 μg/kg). Evaluated parameters included: tidal volume, respiratory rate, minute volume, inspiratory time, expiratory time, inspiratory:expiratory ratio, peak inspiratory flow, peak expiratory flow, time to peak expiratory flow, mid-tidal expiratory flow (EF50) and inspiratory to total breath ratio. After methacholine high dose, expiratory time was significantly increased between 65 and 160 min after dosing while inspiratory time was increased between 130 and 150 min after administration. After albuterol, expiration time was significantly longer between 55 and 70 min post-treatment and at 85 and 135 min post-treatment. EF50 was decreased with both bronchoconstriction and bronchodilation. In conclusion, inspiratory time, expiratory time and EF50 appear to be sensitive and useful parameters to evaluate airway changes and could increase sensitivity of respiratory safety models to satisfy the regulatory guideline S7A.

212 RESPIRATORY SAFETY PHARMACOLOGY: COMPARISON OF CONSCIOUS BEAGLE DOGS, CYNOMOLGUS MONKEY AND SPRAGUE-DAWLEY RATS.

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Sprague-Dawley rats are often used to fulfill regulatory requirement for respiratory safety testing under S7A. When the rat is not considered to be a relevant species, Beagle dogs or Cynomolgus monkeys are sometimes used. The 3 species present different physiological characteristics that could affect the sensitivity of adverse respiratory effects detection. The current analysis evaluated the sensitivity and the clinical relevance of each of the three models. To achieve study goals, all animals were administered a bolus of saline IV (0.02 mL/kg) and equivalent doses (based on allometric conversion) of positive control drugs. Rats, (n=8), dogs (n=5 to 12) and monkeys (n=7) received inhaled albuterol (Rats: 0.4 mg/kg; Dogs 0.1 mg/kg; Monkeys 0.1 and 0.2 mg/kg), IV methacholine (Rats: 28 and 136 μg/kg; Dogs: 2 and 8 μg/kg; Monkeys 3.4, 13.5 and 68 μg/kg) and IV remifentanil (Rats 28 μg/kg; Dogs 4 μg/kg; Monkeys 6.75 μg/kg). Tidal volume (TV), respiratory rate (RR) and minute ventilation (MV) were recorded continuously with head-out plethysmographs, masks and bias flow helmets in rats, dogs and monkeys, respectively. Average baseline TV was 5.5, 16.7 and 12.0 mL/kg for rats, dogs and monkeys, respectively. Albuterol induced a biphasic increase in MV in monkeys with an initial increase in TV followed by an increase in RR. Both rats and dogs presented a significant increase in TV. For remifentanil, apnea was noted in all except 1 monkey while dogs and rats did not present apnea at equivalent dose. Methacholine induced a slight increase in MV in rats while a more important increase was noted in dogs. Only monkeys presented a decrease in MV at high doses which is the expected response in healthy human volunteers. Based on these results, all 3 species are considered adequate for respiratory safety pharmacology evaluations. The rat was the less sensitive model most likely due to the less developed respiratory system in this species. Monkeys presented drug responses closest to human responses.

213 OPTIMIZATION OF ACCLIMATION AND EXPERIMENTAL PROCEDURES FOR HEAD-ONLY INHALATION EXPOSURE OF TRIAMCINOLONE ACETONIDE IN CYNOMOLGUS MONKEYS.

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The inhalation system for Cynomolgus monkeys consists of a paired exposure helmet and in-house designed restraint chair. Animals scheduled for use in a 28-day inhalation toxicity study were acclimated to the exposure system over a 5-week period (3 sessions/week, up to 2 hours/session). Successful training of 16/19 males and 18/19 females was demonstrated using behavioral observations and minute volume (MV) measurements. The mean MV values were 0.52 and 0.48 L/min./kg body weight for males and females, respectively. Exposure methods were tested in a separate 14-day inhalation study of the corticosteroid drug, triamcinolone acetonide. Groups of 3 male Cynomolgus monkeys were exposed daily to mean analyzed powder aerosol concentrations of 0, 39 and 104 μg/L. Based on measured MV values, the overall mean estimated inhaled doses were 0, 518 ± 188.2 and 2053 ± 892.8 μg/kg/day. Toxicologic endpoints consisted of clinical observations, body weights, standard serum chemistry and hematology parameters, macroscopic observations, selected organ weights at necropsy and microscopic examination of selected tissues. Body weights, clinical observations, serum chemistry and hematology parameters and gross necropsy observations were unaffected by test article exposure. Lower organ weights in the thymus, adrenal glands, lungs and testes appeared to be test article-related with corticosteroid dependence likely for the thymus and adrenal glands. Differences from control group means were 37% and 46% for the thymus and 1.5% (increase) and 12% for the adrenal glands in the low- and high-dose groups, respectively. An expected corticosteroid-related microscopic finding, atrophy of the thymus, was observed in 1 of 3 low-dose animals and 2 of 3 high-dose animals. Increased alveolar macrophage numbers in the lungs of some treated animals were also noted during microscopic examination.

214 MICROARRAY ANALYSIS OF MURINE LUNGS FOLLOWING PULMONARY EXPOSURE TO DIESEL EXHAUST PARTICLES.

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Diesel exhaust particles (DEP), main constituents of PM2.5 in urban areas, affect a variety of respiratory diseases. The present study analyzed the gene expression in the murine lungs using microarray analysis in each pathologic stage of the DEP-induced inflammatory lung injury. Male ICR mice (6wks) were intratracheally administrated DEP (100 μg/body) once a week for 6 weeks. The gene expression in the murine lungs was analyzed 4 or 24 hours after each instillation at 0, 2, 4, or 6weeks (=DEP administration; 1, 3, 5, or 7 times) with GeneChip Mouse Expression 430A (Affymetrix). The number of 2-fold up-regulated genes following to DEP exposure as compared to vehicle exposure showed a time-dependent increase in overall trend. In addition, the number of up-regulated genes was much greater at 4 hours after each instillation than at 24 hours. On the other hand, 0.5-fold down-regulated genes were scarcely detected at 4 or 24 hours after the instillation. Functional analyses showed up-regulation of oxidative stress-related genes such as heme oxygenase 1, metallothionein 1, and metallothionein 2, and drug metabolism-related genes such as cytochrome P450, family 1, subfamily a, polypeptide 1 and cytochrome P450, family 1, subfamily b, polypeptide 1, 4 hours after each instillation. In contrast, serum amyloid A 3 and lipocalin 2, proinflammatory molecules, were enhanced at 24 hours after each administration. Taken together, pulmonary exposure to DEP causes a time-dependent increase in the number of up-regulated genes such as antioxidants, drug metabolic enzymes, and proinflammatory molecules, which should be critical in the development and progression of and/or protection against inflammatory lung injury. Furthermore, microarray analysis should be effective for the determination of sensitive biomarkers and pathways involved in the health effects of the exposure to environmental chemicals, e.g., DEP.

215 NOSE-ONLY INHALATION EXPOSURE CAN DEPLETE LIVER GLUTATHIONE LEVELS.

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Nose-only inhalation is a widely accepted method for exposing small mammals to potential toxicants. One drawback of this method, however, is that it requires the animals to be physically restrained; a procedure that may produce stress. As part of an experiment to evaluate the toxicity of complex hydrocarbon mixtures (JP-8 jet fuel), we assessed glutathione levels in liver and other organs of rats receiving nose-only and whole body exposures to fuel and to air under conditions of anaesthesia and normal consciousness. The objectives of this study were to determine whether or not JP-8 exposure would deplete glutathione in vivo as it does in vitro and also to determine whether or not nose-only exposure by itself would alter glutathione levels. Rats were exposed to JP-8 jet fuel (1000mg/m3) and to air for 4 hr under conditions of pentobarbital anaesthesia or in a fully conscious state in a commercial nose-only exposure system. Subsequently, the rats were euthanized so that tissue could be harvested for total glutathione level in liver, brain and lung. Glutathione levels were significantly reduced in the liver of rats that were exposed for a 4 hr period in the nose-only exposure equipment. There were no differences in the extent of liver glutathione levels among conscious rats receiving JP-8 fuel or air. By contrast, glutathione levels remained normal in pentobarbital-anaesthetized rats that received nose-only exposure to JP-8 and to air. Glutathione levels from brain and lung were unchanged for all treatment groups. The data suggest that some aspect of nose-only exposure such as stress is responsible for depleting liver glutathione level independent of the toxic agent used for the exposure. This result identifies a potentially serious confounding variable inherent in nose-only inhalation exposure that calls both for the use of appropriate matched exposures of control subjects as well as recognition that exposure per se may have significant effects related to withstanding oxidative stress. (Supported by the VA- RR&D Service grants #C3575R and C4613L and by the American Petroleum Institute).

216 BISPHENOL A (BPA) DOES NOT EXACERBATE IGE-MEDIATED ASTHMA WHEN EVALUATED IN A RELEVANT MOUSE EXPOSURE MODEL.

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It is well established that the number of people with asthma is increasing in the industrialized world. Various theories for this increase have been proposed. The "hygenic hypothesis" suggests that the increased standard of living in industrialized countries results in decreased exposure to pathogens seen in underdeveloped countries. If pathogens are not present then the immune system may target other proteins to develop antibodies. We hypothesized that the increase in IgE-mediated asthma was also the result of exposure to compounds in the environment. Our studies have focused on the endocrine disrupting compound (ED), BPA, which is a key constituent of polycarbonate and epoxy resins used for food contact applications. In order to test whether BPA had the potential to exacerbate asthma it was necessary to develop an exposure model of asthma relevant to human exposure. Female B6C3F1 mice received one sensitizing dose of ovalbumin (OVA) (0.5, 5, or 50 µg/mouse) on days 1, 3, & 10 and were sacrificed on Day 11 or 15. The model displayed the classic hallmarks and indicators of asthma including OVA-specific serum IgE, bronchoconstriction and airway hyperresponsiveness following challenge with methacholine and eosinophilic inflammation as determined by increased eosinophil peroxidase. In addition to OVA, mice were exposed to vehicle or one concentration of BPA (12.5, 25, or 50 mg/kg) i.p. EOD for 14 days. In contrast to the results observed by others, in which the sensitizing antigen was administered parenterally in conjunction with an adjuvant, treatment with BPA had no effects on antigen-specific serum IgE production, eosinophilia, or airway hyperresponsiveness. The use of antigens administered in combination with adjuvants to sensitize and initiate an asthma-like response, may result in compounds being labeled as asthma exacerbating chemicals, when in actuality the increased effects observed are not related to the compound of interest, but are artifacts of the route of sensitization and the use of adjuvants in the animal models. (Supported in part by NIEHS Contract ES 05454.)

217 IS TOLUENE DIAMINE (TDA) A SENSITIZER AND IS THERE CROSS-REACTIVITY BETWEEN TOLUENE DIAMINE (TDA) AND TOLUENE DIISOCYANATE (TDI)?

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Toluene diamine (TDA) is formed when toluene diisocyanate (TDI), a potent sensitizer, comes in contact with aqueous environment. The sensitizing capacity of TDA and the cross-reactivity between TDI and TDA are unknown. TDA (5-25%) and TDI (0.3%), dissolved in acetone/olive oil (AOO) (4:1) were tested in the mouse local lymph node assay (LLNA). To determine the respiratory sensitizing capacity of TDA and the cross-reaction with TDI, a mouse model of chemical-induced asthma was used. On day 1 and 8, BALB/C mice were sensitized with TDI (0.3%), TDA (20%) or AOO (4:1) on each ear. On day 15, they received an intranasal instillation of TDI (0.1%), TDA (0.5%) or AOO (3:2) and the ventilatory function (Penh) was monitored for 40 min after intranasal challenge. Airway reactivity to methacholine (AHR) was measured (Penh and pulmonary resistance (R)) 46 h later. Total and differential cell counts, TNF-α and MIP-2, were assessed in bronchoalveolar lavage (BAL). Total serum IgE, IgG1 and IgG2a levels were measured. Relative lymphocyte subpopulations (CD4+, CD8+, CD25+ and CD19+) in auricular and superficial cervical lymph nodes and release of IL-4 and IFN-γ by lymphocytes were assessed. The EC3 of TDA in the LLNA is 16.2%. In the mouse model of chemical-induced asthma, only TDI induced a ventilatory response (increased Penh after challenge; increased AHR) inflammatory changes (BAL neutrophils) and immunological changes (increased CD19+ lymphocytes, IL-4 and total serum IgE). TDA did not show a response. Mice sensitized with TDI and challenged with TDA also did not show any airway or inflammatory response, although they had increased levels of total serum IgE. Mice sensitized with TDA and challenged with TDI did not show any response. According to the classification of sensitizers in the LLNA, TDA is a weak dermal sensitizer. In the mouse model of chemical-induced asthma, TDA does not act as a respiratory sensitizer, at the concentration used. No cross-reactivity between TDI and TDA was found.

218 KNOCKDOWN OF GAMMA-GLUTAMYLCYSTEINE SYNTHETASE IN RAT CAUSES ACETAMINOPHEN-INDUCED HEPATOTOXICITY.

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Drug-induced hepatotoxicity is mainly caused by hepatic glutathione (GSH) depletion. In general, the activity of rodent glutathione S-transferase is 10 to 20 times higher than that of humans, which could make the prediction of drug-induced hepatotoxicity in human more difficult. The gamma-glutamylcysteine synthetase (GCS) mainly regulates de novo synthesis of GSH in mammalian cells and plays a central role in antioxidant capacity of cells. Therefore, a GSH-deficient animal model would be useful for the prediction of drug-induced hepatotoxicity. Gamma-GCS is a heterodimer consisting of a light regulatory subunit (GCSl) and a heavy catalytic subunit (GCSh) and catalyzes the rate-limiting step of GSH biosynthesis. An adenovirus vector with short hairpin RNA against rat GCSh (AdGCSh-shRNA) was constructed and used to knockdown the GCSh. H4IIE cells, rat liver hepatocarcinoma, were infected with AdGCSh-shRNA that was efficiently decreased the endogenous levels of mina, protein of GCSh and GSH concentration. In the CYP3A4 overespressed H4IIE cells by the treatment with a recombinant adenovirus encoding CYP3A4, the cytotoxicity of troglitazone was increased by the infection with AdGCSh-shRNA. In conclusion, the inhibition of gamma-Gcs expression by AdGCSh-shRNA was similar to that by BSO in CYP3A4 overexpressed H4IIE cells, suggesting that the present method would be useful to evaluate the cytotoxicity caused by CYP3A4 mediated bioactivation. infection. In the in vivo study in rat, the hepatic GSH level was decreased by 80% 14 days after a single dose of AdGCSh-shRNA (2 x 1011 PFU/ml/body) and this depletion continued for at least 2 weeks. Using this GSH knockdown rat model, APAP-induced hepatotoxicity was shown to be significantly potentiated compared with normal rats. This is the first report of a GSH knockdown rat model, which could be useful for highly sensitive tests of acute and subacute toxicity for drug candidates in preclinical drug development.

219 GENE EXPRESSION IN VARIOUS BIOLOGICAL PATHWAYS IN FLUTAMIDE-TREATED RATS AND CHIMERIC MICE WITH HUMANIZED LIVER.

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Chimeric PXB mouse, in which more than 70% of hepatic parenchymal cells are replaced by human hepatocytes, is a unique animal model to mimic human-type drug metabolism. This animal model has, therefore, a potential to explain the difference of drug-induced hepatotoxicity in rodent and human. Flutamide, which occasionally causes human-specific idiosyncratic hepatotoxicity, was selected as the test substance to examine changes in hepatic gene expression in rats and PXB mice. After a single oral administration of 300mg/kg flutamide or vehicle control to rats and PXB mice, total RNA fraction was separated from the liver. Hepatic gene expression profiles were then analyzed by using rat-based and human-based oligonucleotide microarray platforms, respectively. Flutamide-induced changes in gene expression were analyzed in each of various biological pathways such as "nuclear receptor mediated gene expression", "cell cycle and apoptosis", "metabolic reactions", etc. In nuclear receptor mediated gene expression such aryl hydrocarbon receptor signaling or NRF2 mediated oxidative stress response, genes responsible for xenobiotics metabolism were strongly induced in flutamide-treated rats. Genes responsible for refolding and/or breaking damaged proteins were also induced strongly in flutamide-treated rats. However, induction of such genes was very weak in flutamide-treated PXB mice. In apoptotic pathway, gene expression profiles in flutamide-treated rats and PXB mice were in an inverse relationship. These results suggest species difference of flutamide-induced hepatocelluar injury and its regeneration processes in rodent and human.

220 INFLAMMATION-MEDIATED LIVER INJURY IN RATS COTREATED WITH SULINDAC AND LIPOPOLYSACCHARIDE.

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Sulindac (SLD) is a nonsteroidal anti-inflammatory drug (NSAID) that has been associated with a greater incidence of idiosyncratic hepatotoxicity than other NSAIDs. Previous studies suggested that lipopolysaccharide (LPS)-induced inflammation potentiates several drug-induced liver injuries in rats or mice. In this study, we tested the hypothesis that SLD/LPS cotreatment causes liver injury in rats. SLD (50mg/kg) or its vehicle was administered to rats by gavage 15.5 hrs before LPS (8.3X105EU/kg) or its saline vehicle (i.v.). Thirty minutes after LPS, SLD or vehicle administration was repeated. Rats were killed at various times after treatment, and serum, plasma and liver samples were taken. Neither SLD alone (one or two administrations) nor LPS alone caused liver injury. Cotreatment with SLD/LPS led to increased activity of alanine aminotransferase (ALT) in serum and histological evidence of liver damage. TNF-a concentration in plasma was increased by LPS ir-

respective of cotreatment with SLD. Accumulation of neutrophils (PMNs) in the liver was greater in SLD/LPS-cotreated rats compared to all other groups. Etanercept, a soluble TNF-a receptor, reduced SLD/LPS-induced liver injury. Liver injury was also attenuated by prior depletion of PMNs or by pretreatment with the PMN protease inhibitor, eglin C. These results suggest that TNF-a and PMNs and their proteases play important roles in SLD/LPS-induced liver injury. (Supported by NIH GMO75865.)

221 MODIFIED ALCOHOL LIQUID DIET MODEL FOR LIVER INJURY IN MICE.

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Alcohol is a known hepato-toxicant and both pathological features and the molecular mechanism of liver injury are thought to be similar in rodents and humans. The enteral alcohol feeding model, first developed for rats and later for mice, produces injury similar to that found in human patients who abuse alcohol. The advantages of the enteral model over ad lib liquid diet models include continuous delivery of alcohol 24 hours a day which simulates binge drinking, robustness of the steato-hepatitis phenotype, and a characteristic cycling in urine alcohol concentrations due to diurnal variation in basal metabolism and hormones is seen with high corn oil based diet. Traditional ad lib feeding with mixed fats and alcohol causes only fatty liver, but is widely used due to the ease of handling. The purpose of this experiment was to determine if ad lib feeding of liquid diet using corn oil as the source of fat would produce a liver injury phenotype similar to human disease. Male C57Bl/6J mice were fed isocaloric control or ethanol-containing liquid diets (Dyets, Bethlehem, PA; Cat # 710034) for 4 weeks. Mice were gradually adapted to increasing concentrations of ethanol in the diet until they were able to tolerate the full dose of ~30 g/kg/day ethanol. After 4 wks, blood alcohol levels (281.6±32.5 mg/dl) and serum ALT (74.3±18 U/l) were significantly elevated. Liver histopathology was evaluated in a blind manner and showed that a total liver pathology score (a sum of scores for steatosis, inflammation and necrosis) was significantly greater in ethanol-fed mice (4.13±0.1) as compared to controls (0.63 + 0.3). These data are consistent with published liver injury phenotypes for the enteral alcohol feeding model. Since our modified alcohol liquid diet model is comparable in phenotype to the enteral model yet is much easier to manage, it may be used for studies of alcohol-induced liver injury by the laboratories that lack surgical expertise required to conduct enteral feeding. (Supported by AA016258)

222 A NOVEL "PATIENT-LIKE" MODEL OF CHOLANGIOCARCINOMA PROGRESSION BASED ON BILE DUCT INOCULATION OF TRANSFORMED RAT CHOLANGIOCYTES OVEREXPRESSING ERBB2.

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Intrahepatic cholangiocarcinoma, because of its increasing incidence and high rates of mortality, presents an important biomedical challenge. In an effort to recapitulate key biological and clinical features of the progressive human disease, we have established a novel preclinical rat model based on bile duct inoculation of a tumorigenic rat cholangiocyte cell line, designated BDEneu, that was previously developed in our laboratory (Lai, G.-H. et al., Gastroenterology 2005, 129:2047-2057). BDEneu cells overexpress rat *neu* (*erbB-2*) oncogene, exhibit up-regulation of epidermal growth factor receptor, and overexpress cyclooxygenase-2 when compared with non-tumorigenic control rat cholangiocytes. Inoculation of 4 x 10^6 BDEneu cells with a greater than 90% viability into the proximal bile duct of isogenic Fischer 344 male rats produced over a 21 to 26 day period a 100% incidence of moderately differentiated, invasive cholangiocarcinomas overexpressing ErbB2 autophosphorylated at tyrosine 1248. A time course study further revealed an exponential tumor growth pattern in which tumors detected in liver at ten days after initial cell inoculation had a mean wet weight of 0.7 ± 0.33 g, whereas those at 26 days weighed 16.8 ± 0.26 g. Tumors formed at 21-26 days largely replaced the left lateral/median hepatic lobes and obstructed bile ducts at the liver hilus. Notably, no gross peritoneal metastases were detected at the 10 day time point, but were prominent at the 21-26 day time periods. The marked increase in liver tumor growth together with associated peritoneal metastases paralleled tumor-induced biliary obstruction, as reflected by a progressive increase in serum bilirubin levels and the development of icteric liver. These results support a novel model of cholangiocarcinoma mimicking progressive development of the advanced human disease, and may serve as a powerful preclinical platform for rapid *in vivo* testing of novel treatments for cholangiocarcinoma. (Supported by R01 CA 083650 and R01 CA 039225 to A.E.S.).

223 MONITORING A MOUSE WHICH EXPRESSES A YELLOW FLUORESCENT PROTEIN IN MONOCYTES WITH INTRAVITAL MICROSCOPY.

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Background: Leukocyte-endothelial cell adhesion through specific receptor-ligand interactions is an early step in inflammatory disorders of the vasculature. Intravital microscopic techniques allow for real-time evaluation of these interactions in a live animal. In this report, we describe the use of this technique in a transgenic mouse model expressing yellow fluorescent protein (YFP) under the control of a monocyte specific promoter c-fms (Csf1r). Methods: Transgenic mice (c fmsYFP+/+, n=8) at 8-12 weeks were anesthetized and the venules in the cremaster muscle evaluated by intravital fluorescent microscopy. Blood was collected and expression of YFP in peripheral leukocytes was measured. Intravital microscopy in c fmsYFP+/+ was compared with in vivo Rhodamine 6G labeling of leukocytes as well as with mice transplanted with marrow derived from mice expressing the green fluorescent protein (GFP). To assess the role of a prototypical pro-inflammatory agent that promotes leukocyte adhesion, TNFα (1μg/kg) was administered followed by determination of leukocyte adhesion, rolling, and local shear stress 4 hours later. Results: 33% of peripheral white blood cells (WBC) of the c fmsYFP+/+ mice expressed the YFP protein. 90% of monocytes (CD14+) were YFP+, c fmsYFP+/+ mice provided accurate labeling of monocytes compared with other non-selective approaches such as Rhodamine-6G labeling or GFP marrow-transplanted mice. TNFα at a dose of 1μg/kg resulted in a five fold increase in adhesion of YFP+ cells to the endothelium suggesting preserved responses to this agent along with expected relationships between shear stress and monocyte adhesion. Conclusions: c fmsYFP+/+ mouse represents a useful model to monocyte endothelial interactions in-vivo and to study the contribution of this cell type in pathophysiology.

224 LIVER-SELECTIVE EXPRESSION OF FUNCTIONAL HUMAN ARYLAMINE ACETYLTRANSFERASE NAT2 IN TRANSGENIC MICE.

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Models to explain the tissue-selective toxicity of aromatic amines must account for tissue-selective expression the enzymes capable of detoxification and metabolic activation of these chemicals. Human NAT2 displays liver-selective expression, in contrast to the ubiquitous tissue expression of NAT1. To facilitate investigations into the role of liver-selective NAT2 expression in modulating aromatic amine disposition and toxicity, we developed a transgenic mouse model that expresses human NAT2 in a liver-selective fashion. Preliminary promoter analysis experiments indicated that both the human NAT2 gene promoter region and the mouse albumin gene promoter/enhancer region are activated in a similar fashion by the liver-enriched transcription factors C/EBPα and DBP. Thus we linked the human NAT2 gene's protein-coding region to a 2.3 kb segment of the mouse albumin promoter/enhancer, injected this hNAT2-alb transgene construct into mouse oocytes, and tested animals derived from implanted oocytes for transgene incorporation by Southern blotting. We obtained a single transgenic founder animal that possessed one copy of the human NAT2 coding region. Hemizygous offspring were characterized with respect to human NAT2 expression using the NAT2-selective substrate sulfamethazine (SMZ) as a substrate for in vitro enzyme assays. SMZ-NAT activity was present in liver cytosols from transgenic mice at a 10-fold higher level than that observed in wild-type controls, and was comparable to that in livers from human NAT2 'rapid acetylators'. SMZ-NAT activity in kidney from transgenic mice was no different than non-transgenic animals, and that in transgenic colon was modestly (~2-fold) increased over non-transgenics. We are currently deriving human liver-selective NAT2 transgenic mice upon a mouse Nat1/Nat2 null background. These mice will be a valuable mechanistic and predictive tool for investigating the role of liver NAT2 in the biotransformation and toxicity of aromatic amines in humans.

225 TOXICOLOGICAL RESPONSES TO 1, 2-DICHLORO-4-NITROBENZENE IN GLUTATHIONE S-TRANSFERASE M1 KNOCKOUT MICE.

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Glutathione S-transferase (GST) is a major phase II enzyme that catalyzes the conjugation and detoxification of electrophilic compounds with glutathione. However, it has been difficult to reveal the contribution of each GST isoform due to the existence of its various isoforms. To simplify the complicated contribution of GST isoforms and investigate the usefulness of knockout mice for toxicity studies, we generated GSTM1 knockout (GSTM1 KO) mice and characterized the phenotypes by

in vitro and *in vivo* metabolic studies. The effect of GSTM1 disruption on toxicological response was demonstrated in single and 14-day repeated dose toxicity studies by the dosing of 1,2-dichloro-4-nitrobenzene (DCNB), a specific substrate to GSTM class. The *in vitro* GST activities to DCNB in cytosol prepared from liver and kidney were much lower in the GSTM1 KO mice than in the wild-type mice. Single oral administration of DCNB at 100 mg/kg to the wild-type and GSTM1 KO mice resulted in remarkably high plasma concentrations of DCNB in the GSTM1 KO mice. According to the results of a single dose toxicity study at 500 and 1000 mg/kg of DCNB, marked increase in blood methemoglobin was observed in the GSTM1 KO mice but not in the wild-type mice. Therefore, GSTM1 KO mice were considered to be sensitive to the methemoglobinemia induced by a single dosing of DCNB. In a 14-day repeated dose toxicity study at 150, 300 and 600 mg/kg of DCNB, marked increase in blood methemoglobin was observed in both the wild-type and GSTM1 KO mice. However, the increases in blood reticulocyte, spleen weight and extramedullary hematopoiesis in the spleen were markedly observed only in the GSTM1 KO mice.
In conclusion, GSTM1 KO mice markedly showed methemoglobinemia and its adaptive responses in toxicity studies, and these findings were considered to be due to high exposure to DCNB resulting from GSTM1 disruption. GSTM1 KO mice would be a useful animal model to evaluate GSTM1 metabolism related toxicity.

226 A NOVEL TRANSGENIC MOUSE MODEL EXPRESSING A HUMAN ENZYME FOR REPAIR OF OXIDATIVE DNA DAMAGE.

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The importance of DNA repair in disease is suggested by recent associations of base excision repair (**BER**) pathway deficiencies with teratogenesis, aging and age-related diseases such as cancer and neurodegeneration. Oxoguanine glycosylase 1 (**OGG1**) is the rate-limiting enzyme in the BER pathway, and is primarily responsible for excision of 8-oxo-2′-deoxyguanosine (**8-oxodG**), a particular oxidative macromolecular lesion implicated in the pathogenicity of various reactive oxygen species (**ROS**)-initiating agents. Accordingly, we engineered a novel BER-enhanced transgenic mouse expressing human α-OGG1 to investigate the role of oxidative DNA damage and repair in ROS-mediated pathologies. Briefly, pC3/pCAGGS-ogg1-FLAG expression vectors were electroporated into murine R1 embryonic stem (**ES**) cells. Candidate ES clones expressing OGG1 were utilized for ES cell aggregations, which were subsequently implanted in to pseudo-pregnant CD1 dams. Chimeric progeny were then mated, and their offspring genotyped by PCR to verify germline transmission of the *α-ogg1-FLAG* transgene. Two independent founder males were derived for each independent transgene insertion event. Expression of the transgene was verified by anti-FLAG immunohistochemistry of major organs, namely the brain and liver. Western analyses of tissue homogenates demonstrated the presence of a single reactive species of the predicated molecular weight. This transgenic mouse model may prove useful in elucidating the pathogenic contributions of oxidative DNA damage, and the role of DNA repair status as a potential risk factor for such ROS-initiated pathologies. (Support: NIEHS R21-ES013848, CIHR)

227 CARCINOGENIC COMPARATIVE STUDY ON CB6F1 TG RASH2 MICE PRODUCED BY TWO BREEDING FACILITIES.

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High carcinogenic sensitivity and reproducibility for human carcinogens in rasH2 mice have been validated by a large number of certification studies (Yamamoto et al., 1998; Usui et al., 2001). At present, this strain of mouse is produced by two breeding facilities, Taconic (Germantown, NY) and CLEA Japan, Inc. (Shizuoka, Japan), and supplied all over the world. To confirm phenotypic conformity of both mice, a 26-week carcinogenicity test was performed on a total of 120 mice including 30 male and female rasH2 mice each obtained from Taconic, and 30 male and female rasH2 mice each obtained from CLEA Japan, Inc. under the same protocol and environmental conditions in our facility. All mice were divided into a vehicle (citrate buffer at pH 4.5, 10 mL/kg, single intraperitoneal injection) group and a MNU (N-methyl-N-nitrosourea, 75 mg/kg, single intraperitoneal injection) group. The survival rate of the vehicle group was maintained at 100% for mice from both facilities at the completion of test. In the MNU group, MNU induced tumor death occurred from 9 to 12 weeks after administration, and the final survival rate for both facilities was 6.7%. In the pathological examination, only benign tumors of lung, spleen, forestomach and skin were observed in a few mice in the vehicle groups of both facilities. In the MNU group, the incidence of forestomach papilloma/squamous cell carcinoma in mice from both facilities was 100%. The incidences of malignant lymphoma in Taconic mice and CLEA Japan, Inc. mice were 93.3% and 86.7%, respectively, and no significant difference was observed (Fisher exact test). Although lung adenoma and skin papilloma/keratoacanthoma were observed in several mice from both facilities, no significant differences were found. Furthermore, data from this test were similar to the background data. Consequently, phenotypic conformity of rasH2 mice derived from two breeding facilities was confirmed by the present study.

228 A NOVEL PARADIGM FOR ASSESSING EFFICACIES OF POTENTIAL ANTIDOTES AGAINST NEUROTOXINS IN MICE.

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Development of antidotes to central nervous system (CNS) poisons has been difficult, in part, because of their extreme toxicity over a small dose range. To address shortcomings with current animal models and ethical guidelines of our IACUC, we devised a new test paradigm based on recovery from a toxic sub-lethal dose of cyanide. The approach minimizes distress, allows maximal survival of the cyanide-treated animals, enhances the power of statistical analysis, and significantly reduces the number of animals required for analysis. A powerful aspect of this approach is that efficacies of novel compounds may be assessed both pre- and post-CN treatment, in contrast to LD50 studies that only allow testing of antidotes pre-CN administration. Administration of Cyanide rapidly disrupts neuromuscular coordination. The end point of the paradigm is recovery of neuromuscular coordination, i.e., the righting reflex (Koplowitz et al., 1989; Baskin et al., 1999;). An effective antidote reduces the recovery period. This model was used to evaluate cyanide toxicity and to evaluate the currently used cyanide antidotes: nitrite-thiosulfate (N/T) and hydroxocobalamin in addition to novel antidotes discovered in our labs. While the current antidotes were effective when administrated pre-CN, they significantly lost efficacy when administered post-CN. The recovery time of mice given the N/T combination at 10 min post-CN was the same as the CN-treated controls. At 20 min post-CN, N/T significantly prolonged recovery time over that of controls by 1.5-fold. Among our novel antidotes, prodrugs that deliver beta-mercaptopyruvate, we have selected candidates for further preclinical development that are superior to the existing clinical antidotes; they can be administered either by injection or orally, and are effective when administered both pre- and post-cyanide.

229 NON-INVASIVE MOUSE GENOTYPING USING DNA ISOLATED FROM FECAL SAMPLES.

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Methods for genotyping mice typically employ collection of tissues by invasive procedures, such as tail clipping, toe amputation, and ear notching, that may require anesthesia and can induce stresses that introduce unwanted variables in subsequent studies of the animals. Further, animal care and use guidelines impose limits on frequency and animal age of sample collection. Mouse feces can be obtained non-invasively and offer a continuously available source of mouse DNA, presumably due to sloughed intestinal epithelial cells. Fecal samples have been used as a source of DNA for genotyping, but methods published to date are confounded by issues of contamination by bacterial DNA and PCR inhibitors and the need for nested PCR. In the present studies, we tested the hypothesis that reliable mouse genotyping from fecal samples can be accomplished by more efficient strategies than have been reported to date. From each unanesthetized mouse, two fecal pellets were collected and washed with 0.3 ml of PBS by gentle flicking for 1 min. The supernatants were removed, the DNA was isolated using magnetic beads (Dynabeads DNA DIRECT Universal), and the samples were amplified by PCR and visualized. In studies with chemically induced homozygous glutathione reductase-deficient knockout mice, C3H/HeN wild type mice, and respective heterozygote mice, bands produced from fecal DNA samples were less intense, but diagnostically equivalent to bands from DNA obtained through traditional phenol/chloroform extractions of tail biopsies of the same mice. Mouse fecal genotyping has minimal impact on the animals and offers important advantages, such as the ability to re-genotype mice, if necessary, and to genotype mice with abnormal wound-healing phenotypes. The fecal DNA sampling method offers important advantages, however, collection of fecal pellets from pups younger than 21 d can be problematic.

230 UNANTICIPATED ANTICOAGULANT RESPONSE TO UNFRACTIONATED HEPARIN IN CYNOMOLGUS MONKEYS MEASURED BY ACTIVATED CLOTTING TIME.

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Several novel anticoagulants are currently under development to address unmet medical needs for patients during cardiac surgery or percutaneous coronary intervention procedures. To maintain hemostasis, the recommended clinical regimen is an intravenous bolus injection of unfractioned heparin (UFH) at 60 U/kg followed by an initial infusion of 12 U/kg/hr. The anticipated response is a prolongation of activated partial thromboplasin time (aPPT) 1.5 to 2.0 fold over baseline (~60 sec). The cynomolgus monkey is widely utilized for evaluating new drugs. To evaluate the response to UFH, a feasibility study was conducted in 2 tethered, jacketed male cynomolgus monkeys surgically implanted with femoral and jugular vein catheters for independent infusion and blood sample collection. On 4 successive days, animals were administered 50 + 10, 100 + 20, 200 + 40 and 300 + 60 IU/kg IV bolus + IU/kg/hr infusion for 1 hr. Activated clotting time (ACT) was measured in vitro and in vivo using the Point-of-Care HEMOCHRON Jr+ Analyzer and disposable cuvettes. For In vitro testing, whole fresh blood (0.5 mL) was mixed 1:1 with IV-bolus UFH dose preparation, immediately transferred to a cuvette and ACT measured. For in vivo testing, whole blood was collected at 10 min post-start of infusion and 10 min post-end of infusion. Blood was immediately transferred to a cuvette for ACT determination. The anticipated change in activated clotting time typically measured in colony animals is approximately a 2.5 fold prolongation (225 sec) over baseline (90 sec).
In vitro results produced the anticipated response with all ACT >400 sec. In vivo results demonstrated that approximately 5-fold more UFH was required than anticipated to produce the expected pharmacologic effect at 10 min post-start of infusion. In conclusion, this pilot study demonstrated that cynomolgus monkeys are notably less responsive to UFH anticoagulant compared to the currently recommended clinical dosages.

231 INTRACELLULAR ACIDIFICATION PROMOTES ALLOXAN-INDUCED TOXICITY IN PANCREATIC ISLETS.

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Alloxan (1,3-diazinane-2,4,5,6-tetrone) is commonly used to induce type I diabetes in rodents. When given intravenously or subcutaneously, alloxan inhibits the sensitivity of the islets to glucose stimulation. However, pretreatment with glucose protects islets through an unknown mechanism. Because alloxan is not stable at elevated pH, and because glucose increases intracellular pH in islets, we hypothesized that protection by glucose against alloxan toxicity is mediated in part by an increase in intracellular pH. To test the effects of intracellular pH on the response of islets to glucose stimulation following treatment with alloxan, we used self-referencing microsensors to measure changes in oxygen flux from intact pancreatic islets isolated from female CD1 mice in response to glucose or alloxan. Treatment of isolated islets with glucose (10 mM) resulted in a marked increase in oxygen flux (2-3 fold) and insulin secretion. Pretreatment with high (1 mM), but not low (100 μM or less), concentrations of alloxan prevented glucose-stimulated oxygen consumption. To test the effect of changes in intracellular pH on alloxan-mediated toxicity, islets were pretreated with amiloride (1 mM), a potassium channel inhibitor that lowers islet cell intracellular pH. We found that amiloride enhanced the susceptibility of islets to alloxan (100 μM) and prevented glucose-stimulated oxygen consumption following alloxan treatment. Amiloride treatment alone, however, did not inhibit glucose-stimulated oxygen consumption or insulin release. Taken together, these data indicate that intracellular pH plays a critical role in the etiology and development of alloxan-mediated diabetes. (Supported by ES005022 and RR001395).

232 THE QUANTIFICATION OF STRUCTURAL/FUNCTIONAL IMPAIRMENT IN THE EXPERIMENTAL DOG OSTEOARTHRITIS MODEL IS A USEFUL TOOL FOR DRUG TESTING.

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Lameness is a cardinal feature of osteoarthritis (OA) and reflects a pain-related functional impairment. The impact of OA structural damages on the dog's gait has been poorly documented. Therefore, this study explored the relationship between structural damages induced in the experimental dog OA model, as assessed by high-field (1.5T) magnetic resonance imaging (MRI), computed radiographs (CR), and limb function over a 26-week period. OA was surgically induced by transection of the right cranial cruciate ligament in 5 dogs. Peak vertical force (PVF) and contact area (CA) were acquired at baseline, 4, 8, and 26 weeks post surgery in parallel to MRI and CR of the dog's right stifle. Osteophytosis, subchondral sclerosis and joint effusion were scored (0-3, each) based on MRI and CR findings. Cartilage defect (0-4), synovial thickening and subchondral bone marrow lesions (BML) (0-3, each) were scored while loss of cartilage volume (mm3) was computed. Clinical signs of OA were scored (0-4). Animals were sacrificed at week 26 and macroscopic and histological evaluations of the joint lesions were performed. Significant level of limb impairment, as well as OA features were detected with MRI and CR. CA significantly correlated with clinical signs of OA and limb impairment was better correlated by PVF. The increase in PVF at week 26 versus week 4 correlated significantly ($P<0.05$) with less severe cartilage defect, osteophytosis, joint effusion and BML and to a lesser extent with cartilage volume loss. The macroscopic and histological evaluations of cartilage lesions and osteophytes at week 26 corresponded to MRI findings. In contrast to MRI, CR findings were poorly correlated with limb function. The combined use of MRI and gait analysis in the experimental dog OA model offers a reliable and innovative tool to assess the evolution of disease and the potential effect of disease modifying OA drugs (DMOAD).

233 TOXICITY OF INTRAVENOUSLY ADMINISTERED ACIDIC AND ALKALINE VEHICLE FORMULATIONS IN MICE.

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The intravenous (i.v.) route of administration of pharmaceuticals is commonly used in preclinical studies to mimic the intended clinical route and/or to increase the compound systemic exposure. Many pharmaceutical agents intended for i.v. administration are insoluble in Vehicle at physiological pH 7.2-7.4 and formulating in acidic or alkaline Vehicle becomes a necessity. The toxicity of acidic and alkaline Vehicles administered via the i.v. route is not well characterized in the scientific literature. Characterizing these effects will provide better understanding of the formulated compound toxicity. This study investigated toxicity of vehicles with wide pH range (2.7-11.5) administered intravenously (slow tail vein bolus injection) to ICR and NMRI out-bred mice (3-20 mice injected/pH formulation) at dose volumes between 5-20 mL/kg. The Vehicle formulations evaluated in the acidic pH range were: Citric acid in saline, pH 2.7-2.8; Lactic acid in dextrose, pH 3.5; Captisol in water, pH 4.5; Hydroxypropylbetacyclodextrin (HPBCD) in citrate buffer, pH 4.9-5.3; Phosphate, Tween 20 and sorbitol, pH 6.0; and Saline, pH 6.1. The Vehicle formulations made in the alkaline pH range contained: Sucrose, Tween 80, saline and tromethamine (TRIS), pH 8.0; Saline, pH 9-9.5; and polyethylene glycol (PEG) in water at pH 9.0, 10.0, 11.0 and 11.5. Animals were observed hourly for up to 6 hours post-injection on Day 1 and during the course of the study to evaluate adverse local and systemic toxicity, including moribundity and mortality. Alkaline Vehicles resulted in local tail irritation in few animals which resolved over 2-3 days. Animals injected with acidic Vehicle formulations showed no signs of local or systemic toxicity. All the test animals survived for up to 15 days post-injection and no mortality occurred in any of the 12 separate studies we conducted. Therefore, both acidic and alkaline pH Vehicle formulations tested in the pH range 2.7-11.5 were considered safe for i.v. dosing in mice.

234 THE LOW DOSE EFFECTS OF PCB126 ON RAT LIVER METABOLISM IN RATS GIVEN A CONTROLLED DIETARY LEVEL OF SELENIUM.

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Previous studies indicated that dioxin-like polychlorinated biphenyls (PCBs) may influence selenium levels, an important component of antioxidant enzymes in the liver. PCB126 (3,3',4,4',5-pentachlorobiphenyl), the most toxic congener of the PCB family, is able to mimic dioxin and bind to the Ah receptor and elicit numerous toxic effects including oxidative stress. We hypothesized that even at a low dose exposure this PCB congener will lower selenium levels in the rat liver, besides inducing changes in hepatic enzymes. Male Sprague-Dawley rats were fed an AIN-93 purified diet with the selenium level controlled at 0.2ppm. Following nine days of acclimatization, animals received a single intraperitoneal injection of the corn oil vehicle (control) or PCB126 (1μmol/kg body weight) in corn oil. The animals were sacrificed two weeks after the injection, and liver, kidney, and blood samples were

taken and analyzed. We observed that liver weights from PCB126-treated rats were significantly larger, and this was accompanied by an over 40-fold increase in hepatic microsomal cytochrome P-450 CYP1A1 activity. Hepatic selenium and glutathione levels were significantly decreased compared to control rats, which correlated with a decrease in selenium-dependent glutathione peroxidase (SeGPx) activity. No significant differences were seen in kidney selenium, copper and zinc levels or in hepatic superoxide dismutase (CuZnSOD) activity, despite a significant increase in copper and decrease in zinc levels in the liver. This controlled dietary study shows that even a single low dose of PCB126 may significantly perturb metal homeostasis and antioxidant and enzyme levels in the liver with yet unknown consequences. (Supported by NIEHS P42 ES013661)

235 COMPARISON OF ELECTROCARDIOGRAPHIC EFFECTS IN NONHUMAN PRIMATES: CONSCIOUS UNRESTRAINED *VERSUS* PHYSICALLY OR CHEMICALLY RESTRAINED.

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International regulatory guidelines require collection of electrocardiograms (ECGs) from nonrodents prior to the first in human exposure to a drug candidate. While this is typically conducted in safety pharmacology (telemetry) studies, this is often inappropriate for large molecules due to their long half life. The guideline states that in this case efforts should be made to acquire safety pharmacology endpoints from toxicology studies. Currently, ECG collection from non-human primates in toxicology studies occurs either under anesthesia or by physical restraint one animal at a time. Both methods have undesirable consequences. Physical restraint stimulates the sympathetic nervous system thereby increasing heart rate, and anesthetics potentially interfere with the test compound. Thus, an alternative method to collect high quality ECGs and from multiple animals at a time is required.
A comparison was made among data collected with three methodologies; chemically restrained, physically restrained, and conscious unrestrained animals (n=8/group). Chemically restrained animals were administered an intramuscular injection of 5 to 25 mg of ketamine HCl/kg of body weight; physically restrained animals were placed on a restraint board. In both methods, the animals were placed in dorsal recumbency and data were collected for a period of at least 30 seconds. Unrestrained animals were equipped with an external jacketed telemetry system. Animals were briefly sedated with ketamine HCl for electrode placement. ECG leads were placed in an approximate Lead II configuration. Jackets designed to house the transmitters were placed on the animals. ECG parameters (heart rate, PR, QRS, QT, and QTc) were evaluated using the DSI-PONEMAH software. While all three methods produced high quality data, the ECG data collected via telemetry resulted in lowest basal heart rates. Based on these results, the external jacketed ECG system produces high quality ECGs from undisturbed animals under more biologically relevant physiologic conditions.

236 THE TOXICITY DATA LANDSCAPE FOR ENVIRONMENTAL CHEMICALS.

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Chemical toxicity information is widely dispersed across multiple sources, which hampers the compilation of data for large sets of chemicals. To address this issue, we are developing ACToR (Aggregated Computational Toxicology Resource), which combines information for millions of chemicals from >150 sources including the EPA, NIH, FDA, and corresponding agencies in Canada, Europe and Japan. The database holds chemical structure; physico-chemical properties; biochemical and cell-based assay data; tabular in vivo data; summary toxicology calls; and URLs to toxicology summaries. An important application is to provide a source of consistent in vivo toxicology data for use in toxicity screening efforts. Tens of thousands of chemicals are in common use but only a few have had significant toxicological evaluation due to the expense of animal testing. An alternative screening and prioritization approach uses in vitro assay "signatures" that can predict in vivo phenotypes. This approach can rapidly screen many chemicals and prioritize "positive" chemicals for further evaluation. ACToR enables the consistent compilation of in vivo toxicology data for many chemicals, which is required for screening signature development and validation. We show results for a set of ~11,000 environmental chemicals to be analyzed as part of the EPA ToxCastTM program. These include High and Medium Production Volume chemicals (HPVs and MPVs), pesticide active and inert ingredients and drinking water contaminants. Currently, the number of chemicals in this set with extensive primary toxicity data is ~1,000. However, approximately 50% of the set have at least limited toxicity summaries. ACToR is based on open source technology and will allow full access both EPA-wide and to the public. This abstract has been reviewed by EPA and approved for publication although it does not necessarily reflect Agency policy.

237 TOXCAST: DEVELOPING PREDICTIVE SIGNATURES OF CHEMICALLY INDUCED TOXICITY.

R. J. Kavlock, D. J. Dix, K. Houck, R. Judson, T. Knudsen, M. Martin, A. Richard and W. Setzer. *U.S. Environmental Protection Agency, Research Triangle Park, NC.*

ToxCast™, the United States Environmental Protection Agency's chemical prioritization research program, is developing methods for utilizing computational chemistry, bioactivity profiling and toxicogenomic data to predict rationally the potential for toxicity and prioritize limited testing resources. In the proof-of-concept phase, ToxCast is evaluating chemicals with an existing, rich toxicological database in order to provide an interpretive context for the high throughput screening (HTS) data. This set of 320 reference chemicals is largely derived from active ingredients in food-use pesticides and represents numerous structural classes and phenotypic outcomes, including tumorigens, developmental and reproductive toxicants, neurotoxicants and immunotoxicants. The goal of the ToxCast program is to develop signatures predictive of in vivo toxicity, based on the combined use of physicochemical properties, the traditional independent variables in structure-activity models, as well as in vitro bioactivity data derived from a broad spectrum of over 400 biochemical, cell-based, or gene expression assays. In later phases of ToxCast, the HTS signatures derived for chemicals with toxicity data gaps will be compared with those of well-characterized chemicals, and significant signature matches will identify priority candidates for further testing in traditional animal bioassays. ToxCast data are being generated through a series of external contracts, and by collaborations within EPA and with the National Institutes of Health Chemical Genomics Center. Development of the supporting chemo-informatic infrastructure and initial results from the proof-of-concept phase are early indicators of success in the ToxCast research program. [This work was reviewed by EPA and approved for publication but does not necessarily reflect official Agency policy.]

238 TOXREFDB: CLASSIFYING TOXCAST PHASE I CHEMICALS UTILIZING STRUCTURED TOXICITY INFORMATION.

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There is an essential need for highly detailed chemical classifications within the ToxCast™ research program. In order to develop predictive models and biological signatures utilizing high-throughput screening (HTS) and in vitro genomic data, relevant toxicity endpoints must first be identified and characterized for EPA's ToxCast Phase I chemicals (www.epa.gov/comptox/toxcast). These classifications are being derived from the output of ToxRefDB, a relational database that stores standardized toxicity information, using structured data mining algorithms including tree-based methods. Classification trees are used to predict membership of an object within a category based on a set of measurements. The source toxicity data in ToxRefDB covers multiple study types, including sub-chronic, developmental, reproductive, chronic, and cancer studies, resulting in a complex and diverse set of toxicity endpoints. The inherent hierarchy between the various study types (e.g., sub-chronic and chronic) and biological endpoints (e.g., clinical chemistry and tissue pathology) provide input parameters and class prediction variables for differentiating and predictive classifiers based solely on the in vivo toxicity data. These hierarchical classifications provide phenotypes and sub-phenotypes inferring mode of action information, enabling stronger mechanistic linkage between ToxCast HTS data and the reference toxicity information being analyzed in ToxRefDB and the Aggregated Computational Toxicology Resource (ACToR) environment. This work was reviewed by EPA and approved for publication but does not necessarily reflect official Agency policy.

239 A COMPUTATIONAL FRAMEWORK FOR SYSTEMS-BASED ANALYSIS OF DEVELOPMENTAL TOXICITY.

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EPA is building capacity for multidimensional evaluation of toxicity using information of chemical properties, genomic responses, and cell-based assays (www.epa.gov/comptox/). The ToxRefDB database is making toxicity data accessi-

ble for correlation with bioactivity profiling data from the ToxCast research program. Proof-of-concept is underway (Phase I) to test the hypothesis that bioactivity signatures correlated with endpoints from traditional animal testing can be used to predict adverse outcome. Here we describe a new database module for developmental toxicity. Our first objective is to implement a semi-automated data-capture routine on ACToR that reaches out to databases and resources using a thesaurus of metadata terms. This identifies study records / publications and uses a string of semi-automated steps to fetch, parse, and format associated data. Manual curation is applied for tracking provenance and to confirm categorization on an initial set of Data Evaluation Records from EPA's Office of Pesticide Programs. Our second objective is to develop a Natural Language Processing (NLP) lexicon that flags records holding developmental data and extracts content with logic-based text mining rules. It will be tested on records for the 320 reference chemicals (mostly pesticides) in over 400 high-throughput screening assays for the Phase-I ToxCast project. Traditional developmental toxicity data are fairly complete for these chemicals, and this NLP approach could accelerate and enhance curation of data into ToxRefDB. Correlation of effects in ToxRefDB will be used to identify appropriate phenotypic triggers for a prenatal developmental toxicity study in rats, based upon results from developmental studies in rabbits and reproductive studies in rats. [This work has been reviewed by EPA and approved for publication but does not necessarily reflect official Agency policy].

240 UNDERSTANDING TOXICITY THROUGH THE ANALYSES OF A WELL DESIGNED TOXML DATABASES.

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Scientists from US FDA CFSAN and CDER have constructed toxicity databases that can be used as the training sets for structure activity relationship analyses within FDA including as part of the review process. These databases use the ToxML standard with controlled vocabularies for, so far, studies in genetic toxicity, subchronic/chronic toxicity, and reproductive and developmental toxicity studies. Student interns trained by FDA scientists entered information from reports submitted to FDA and FDA review memoranda into the databases using the Leadscope ToxML Editor. Following quality control steps by FDA scientists, structure-searchable ToxML databases were built. The interns then used the database to search for chemical relationships among the data that had been submitted to FDA. The submission data were analyzed at the compound class level, providing several interesting observations (e.g., there are no carbamates reported in the CFSAN food additives database, and while more than 20% of the drugs from CDER data contained alkyl and aryl halides, less than 3% are found in food additives). Combining the compound classes with biological endpoints allowed us to compare compound classes across the various endpoints. Profiles of compound classes for various genetic toxicity, subchronic/chronic toxicity, and reproductive and developmental toxicity endpoints are compared in this poster. This database project is funded by the cooperative research and development agreements between FDA and Leadscope, Inc. and Lhasa, Ltd.

241 USING THE COMPARATIVE TOXICOGENOMICS DATABASE TO IDENTIFY CHEMICAL-GENE-DISEASE ASSOCIATIONS: ARSENIC AS A CASE STUDY.

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Sponsor: W. Toscano.

The etiology of many chronic diseases involves interactions between environmental factors and genes that modulate important physiological processes. Understanding interactions between chemicals and genes/proteins will provide insight into the mechanisms of chemical action and factors affecting disease susceptibility. The Comparative Toxicogenomics Database (CTD; http://ctd.mdibl.org) provides integrated and curated data describing associations between chemicals, genes/proteins, and human diseases. CTD integrates data for 59,000 chemicals, 1.2 million gene and protein sequences and their associated Gene Ontology (GO) annotations, 83,000 taxonomic terms and 6,000 human diseases. In addition, scientific curators manually capture interactions between chemicals, genes/proteins and human diseases from the published literature. Currently, more than 40,000 interactions have been manually curated for 2,900 chemicals and 5,500 genes.
To illustrate the scope and application of CTD, we present an analysis of curated data for arsenic compounds. Arsenic represents a major global health problem; however, little is known about its mechanism of action or the extent of associated diseases. CTD contains a robust data set for arsenic describing molecular interactions between 20 different arsenic compounds and 1437 genes/proteins. Of the 1437 genes that interact with arsenic, 408 are associated with 440 unique diseases that may be influenced by arsenic (cancer, skin disease, digestive system diseases, nervous system diseases, and others). These inferred disease associations were compared with known arsenic-induced diseases reported in the literature. The data integration and curation strategy for CTD yields insight into the actions of environmental chemicals and provides a basis for developing hypotheses about environmental effects on molecular networks and human diseases.

242 ADVERSE EFFECTS OF PHARMACEUTICALS: A. CONSTRUCTION OF A RELATIONAL DATABASE OF ADVERSE CARDIOLOGICAL EFFECTS USING FDA ARCHIVES, PHARMAPENDIUM, AND PUBLIC SOURCES.

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The Informatics and Computational Safety Analysis Staff at FDA/CDER has created databases of adverse drug effects data of human and animal toxicology findings linked to drug chemical structure, class, clinical indication, and receptor target. These databases are being used to develop quantitative structure-activity relationship (QSAR) models that predict the adverse effects of drugs (see related poster). The pre-clinical, clinical, and post-marketing observations contained in the database were obtained from: FDA's Spontaneous Reporting System, PharmaPendium software, and public sources (Micromedex Integrated Index). PharmaPendium includes information from FDA drug approval packages, Meyler's Side Effects of Drugs, drug monographs from Mosby's Drug Consult, FDA's Adverse Event Reporting System, and the published literature. The standard MedDRA vocabulary used in PharmaPendium was employed for all adverse endpoints and drug generic names in order to integrate the data from multiple sources. The total human adverse effect database contains ~4000 drug compounds; ~10,000 adverse endpoints; and ~10,000,000 reports. To account for variations in the number of adverse reports due to different patient populations for each drug, exposure was estimated based upon the proportional reporting ratio (PRR) of reports for each drug expressed as a percentage of the total reports in the database. The PRR values were used to identify drugs with significant findings. A detailed breakdown of data from 396,985 reports of cardiological adverse effects for conduction, coronary artery, electrocardiogram, heart failure, myocardial, rate rhythm, palpitations, and valve disorder endpoints is presented.

243 LEVERAGING HISTORICAL INFORMATION IN PRECLINICAL TOXICOLOGY STUDIES: THE HEPATOTOXICITY KNOWLEDGEBASE.

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AstraZeneca is focusing on hepatotoxicity as one of the key toxicities in leading our development of better ways to reduce clinical attrition. Part of the overall hepatotoxicity strategy is the provision and better use of prior knowledge (internal and external) to better aid decision-making. A pilot study was run to develop a Hepatotoxicity Knowledgebase (HKB) that would act as a portal to relevant prior knowledge within AZ (e.g. internal documents).
This project uses textmining technology for knowledge extraction around a clearly defined set of business questions, specified by the steering group of domain experts. Knowledge will be extracted from both structured and unstructured text to build on the learning from historical studies and extract concepts for use in critical decision-making. This will allow: Rapid retrieval of relevant information based on target, compound name and structure similarity and development of additional structured datasets for use in building predictive models or new SAR.
The knowledgebase consists of concepts and domain knowledge extracted and stored within a knowledge framework for retrieval. Assertions in HKB will interface with the liver specific assertions generated by the Safety Intelligence Programmme. The links to the original sources for the concepts will be retained. The impact of HKB will be to allow more rapid and effective retrieval of relevant information and hence improved the speed and quality of decision making around liver injury. In the long term, this should result in reduction of the number of projects carrying unknown/unacceptable issues into the clinic and ultimately a reduction in the attrition of compounds due to hepatotoxicity.

244 QSAR MODELING OF HUMAN LIVER ADVERSE EFFECTS DATABASE USING *k*NN METHOD.

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Drug induced liver adverse drug reactions (ADRs) continue to be a major cause of clinical and post-marketing drug withdrawal. Quantitative structure activity relationship (QSAR) modeling is a promising computational approach that has successfully predicted human ether-a-go-go-related gene (hERG) binding-related ADRs in the heart. While liver toxicity is a major concern for drug safety, there are currently no *in silico* models for human liver ADRs. Liver enzyme activity measurements are widely used to assess possible liver damage by drugs and chemicals and the USFDA has compiled a database of human liver enzyme activity data points from reported ADRs on approximately 500 approved drugs. The database includes five liver enzyme data points and a composite score endpoint. In this study, we used measurements of ALT, AST and composite score end points to construct a binary classification scheme for liver ADRs. First, approximately 200 compounds for each endpoint were selected from the database using similarity searching to decrease the number of non-congeneric inactive compounds and achieve a more balanced 40/60 active/inactive ratio. Second, QSAR models were developed using the *k* nearest neighbor (*k*NN) method and either Dragon or MolConnZ chemical descriptors. Each endpoint dataset was divided into multiple training and test sets and models were considered acceptable if leave-one-out (LOO) cross validated R^2 values were >0.5 for training sets and correlation coefficients were >0.6 for test sets. Rigorous external model validation protocols were used to validate the predictivity of the models, which were found to be highly sensitive (>73%) and specific (>94%). These models may be useful in screening pre-clinical drugs for potential hepatotoxicity in humans, reducing attrition rates and associated costs.

245 TWO-STEP QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIP MODELING OF *IN VIVO* TOXICITY USING *IN VITRO* CYTOTOXICITY DATA.

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The German Center for the Documentation and Validation of Alternative Methods (ZEBET) compiled a database of 347 environmental chemicals with experimental in vitro cytotoxicity IC50 and rodent in vivo LD50 values. To assess the correlation between chemical structure and acute in vitro and in vivo toxicity, we used these data for k Nearest Neighbor (kNN) Quantitative Structure-Activity Relationship (QSAR) modeling. When, the kNN QSAR approach using MolConnZ descriptors was applied to the in vitro cytotoxicity (IC50) for 251 compounds selected randomly as the modeling set, prediction accuracy for the external validation set (40 compounds) was 70%. No correlation was found between the IC50 and LD50 values, and no predictive kNN QSAR models of in vivo toxicity could be developed with the whole dataset. However, when the entire dataset was subdivided into two subsets, one included compounds with high correlation between LD50 and IC50 values (R2>0.9), and the second including compounds with poor IC50/LD50 correlation (outlier set), we were successful in establishing predictive QSAR models as follows. First, we developed a classification QSAR model that accurately discriminated the two classes of compounds using MolConnZ descriptors, with the classification accuracy as high as 72% for the external validation set. Second, several kNN QSAR models were developed only for the outlier set, with both training (Q2) and test (R2) sets' fitness exceeding 0.5. Overall, the two-step QSAR approach achieved a prediction accuracy of 72% for the external validation set (25 compounds). We conclude that considerable improvements can be achieved in correlating in vitro and in vivo toxicity data when the activity-correlation based clustering is applied prior to development of predictive QSAR models. This abstract does not represent US EPA policy.

246 *IN SILICO* STUDIES OF THE TOXCAST CHEMICALS INTERACTING WITH BIOMOLECULAR TARGETS.

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Molecular docking, a structure-based in silico tool for chemical library pre-screening in drug discovery, can be used to explore the potential toxicity of environmental chemicals acting at specific biomolecular targets. In this approach, an approximation of the free energy, ΔG(complex), of the formation of the complex between the environmental chemical and the target is obtained. The results correspond to the equilibrium binding affinity, Ka, of a chemical with a target. By docking many ligands into many targets, a virtual Affinity Fingerprint Matrix (vAFM) is constructed and the structure/ multi-target affinity signatures explored.

The exhaustive docking algorithm in the eHiTS programs (SimBioSys Inc., Toronto, Ontario) was applied to construct a vAFM of a diverse set of ~300 environmental chemicals docked to 18 nuclear receptor (NR) crystal structures (PPAR, ER, PGR, AR, MR, LXR, PXR, CAR, VDR, FXR, GR, MR, ROR, RXR). The data was clustered in chemical/target space and specific clusters were profiled. A molecular basis for differentiating promiscuous, tight-binding ligands from non-binding and selective ligands was developed and the findings were corroborated with current NR literature.

With the same approach, a vAFM of several thousand chemicals, containing known endogenous ligands, therapeutic compounds and 306 ToxCast chemicals is being developed for ~200 ToxCast targets, including NRs, kinases, phosphatases, CYPs and esterases. These in silico results will be coupled with ToxCast in vitro assay data and in vivo data to provide mechanistic insights, and to determine a practical strategy for the use of molecular docking results in a screen for chemical toxicity. [This work was reviewed by EPA and approved for publication but does not necessarily reflect official Agency policy.]

247 APPLICATION OF *IN SILICO* TOXICANT-TARGET APPROACH TO SCREENING A CHEMICAL LIBRARY FOR ESTROGENICITY.

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In many mechanisms for the adverse effects of anthropogenic chemicals a critical and perhaps differential step requires the interaction of the chemical with a biological macromolecule. Where the macromolecular target is a receptor, this interaction may be studied by computationally docking the putative ligand into the receptor binding site. Environmental estrogenicity is an example of a process that can be modeled by this in silico approach.

In this study the capacity of a series of 318 chemicals to bind to the estrogen receptor has been evaluated using three different methods for docking. Each method depends on semi-empirical approaches to evaluate the interaction but varies in these specifics: 1) The method for the discovery of the best possible fit between the putative ligand and the receptor, 2) the determination of the energetics of each fit, 3) the semi-empirical atom based parameterization of the interaction. The data set studied contains 281 chemicals recently evaluated using a single rat uterine ER binding assay. This data set contains chemicals that bind much more weakly than estrogen and non-binders. In addition, 37 known strong binders were added. The protein targets were derived from known rER and hER crystal structures.

The result of the docking calculations is a list of chemicals ordered by their predicted affinity for rER. All of the experimental rER binding chemicals appear in the first 27% of the list but are not ordered by their binding affinity. The choice of demarcation between predicted binding and non-binding chemicals is determined by the balance between false positives and false negatives and will be discussed. These results suggest that this approach has value as a prescreen for setting testing priorities. [This work was reviewed by EPA and approved for publication but does not necessarily reflect official Agency policy.]

248 A QSTR ANALYSIS OF CWAS USING COMMERCIAL QSTR MODELS: DEREK AND TOPKAT.

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The risk-assessment (RA) paradigm as defined by the National Academy of Sciences (1983) is comprised of four elements: Hazard Assessment, Dose-response assessment, Exposure Assessment, and Risk Characterization. In order to fill the common experimental data gaps that occur commonly in the RA process, US EPA's National Homeland Security Research Center (NHSRC) is using various commercially available and de novo QSTR models to fill these data gaps. In this study, two commercial QSTR models, DEREK and TOPKAT, were used to predict a number of qualitative and quantitative end points to develop a weight-of-evidence (WOE) approach for a select group of chemical warfare agents (CWAs). The CWAs were assessed for mutagenicity, carcinogenicity, and skin sensitization using DEREK. The chemicals were also assessed for carcinogenicity (NTP), Ames mutagenicity, developmental toxicity potential (DTP), chronic-rat LOAEL (lowest-observed adverse effect level), and skin sensitization NEG vs. SENS using TOPKAT.

DEREK results include: 1) mutagenicity- 60% not computed, 37% plausible, 3% equivocal; 2) carcinogencity-90% not computed, 6% probable, 3% plausible; and 3) skin sensitization-67% not computed, 33% plausible. TOPKAT results include: 1) mutagenicity-23% outside optimum prediction space (OPS), 27% not computed, 37% positive, 13% negative; 2) carcinogenicity-27% outside OPS, 27% not computed, 30% equivocal, 20% positive; 3) skin sensitization-53% outside OPS,

27% not computed, 10% equivocal, 10% positive; 4) developmental toxicity-53% outside OPS, 27% not computed, 10% equivocal, 10% positive; and 5) chronic-rat LOAEL 50% outside OPS, 27% not computed, 23% predicted.
In general, the overall assessment of CWAs by both DEREK and TOPKAT models was rather poor. Consequently, both software programs will need to be updated to include more alerts and fragments to enhance valid predictions for CWAs and other chemicals.

249 ADVERSE EFFECTS OF PHARMACEUTICALS: B. PREDICTION OF ADVERSE CARDIOLOGICAL EFFECTS USING MC4PC, LEADSCOPE, BIOEPISTEME, AND MDL-QSAR SOFTWARE PROGRAMS.

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The Informatics and Computational Safety Analysis Staff at FDA/CDER employed in silico methods to predict possible adverse cardiological effects of drugs in humans. The investigation used a new adverse effect database (see related poster) and quantitative structure-activity relationship (QSAR) models that were constructed using the MC4PC (MultiCASE), BioEpisteme (Prous Institute for Biomedical Research), Leadscope Predictive Data Miner (Leadscope), and MDL-QSAR (MDL Information Systems) programs. Significant adverse cardiological effects were identified using a weight-of-evidence paradigm that employed proportional reporting ratio values of adverse effects to estimate patient population exposure, and contingency table statistics to identify toxicologically related adverse effect endpoints. Performance results are presented for QSAR models predicting eight adverse cardiological endpoints based upon a training data set of 1660 chemicals. Model performance was affected by the ratio of active to inactive (A/I) drugs and models were optimized by adjusting this parameter. QSAR models from individual programs all exhibited high specificity (>80%), high coverage (>90%), but low sensitivity (~40%). However, the four QSAR programs were demonstrated to have complementary adverse effect predictions with increased sensitivity obtained by combining predictions from pairs of programs. Consensus-positive predictions of adverse effects obtained using two or more programs resulted in high confidence estimates of potential drug related cardiological injury.

250 GENERATION AND VALIDATION OF MULTI-GENE VALIDATED BIOMARKERS FOR PREDICTION AND DIAGNOSIS OF BILE DUCT HYPERPLASIA IN RATS.

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Comprehensive mining of large gene expression datasets using sparse linear programming (SPLP) supervised classification methods has yielded numerous high-performing multi-gene biomarkers (signatures) for a variety of pathological and pharmacological endpoints. Defined rules have been developed to allow automated assignment of experiments to signature training sets, facilitating systematic mining of the data. In order to better understand the factors underlying the performance of multi-gene biomarkers, we performed forward validation of previously reported diagnostic and predictive (based on early time point expression data) signatures for toxicant-induced bile duct hyperplasia (BDH). For the signature validation study, rats were treated for 1, 5, or 28 days with compounds not used in the training sets of the existing BDH signatures. Liver gene expression from the 1 and 5 day time points was profiled on CodeLink™ RU1 microarrays and liver histopathology was assessed for all time points. These data were used to forward validate the two BDH signatures and evaluate alternative strategies for signature definition. By increasing the stringency of the histopathological score defining the positive training set class and using an adaptive SPLP algorithm that allows flexibility in signature specificity and sensitivity tolerances, we were able to dramatically improve the performance of the signatures. Sensitivity increased from 25% and 43% for the diagnostic and predictive signatures respectively to 71% and 86% based on forward validation. Specificity increased from 93% to 100% for the diagnostic signature and slightly decreased from 100% to 83% for the predictive signature. Interestingly, the improved diagnostic signature had excellent predictive properties, identifying 7 out of 11 (64%) 5-day treatments that resulted in BDH only after 28 days. When the data obtained from the new study were incorporated into the training sets, estimated performance was increased even further.

251 THIRD-PARTY BIOMARKER VALIDATION AND HYPOTHESIS GENERATION IN A VERY LARGE TOXICOGENOMIC DATABASE.

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One of the most common shortcomings of microarray gene expression studies is small sample size, leading to difficulty in validation of results and overestimates of their power and reproducibility. Expense frequently prohibits a large-enough study, and validation using third-party data sets can be very difficult or unclear due to incompatibilities in technology and experimental design. Addressing both of these problems, we have implemented the "connectivity map" algorithm of Lamb, et al. (2006) on a very large, integrated toxicogenomics database (Ganter et al., 2005). The nonparametric algorithm facilitates evaluation of third-party gene expression biomarkers in the context of a rich and consistent data set, supporting both validation and biological hypothesis generation. As an example, we evaluate a third-party, gene expression-based biomarker for hepatotoxicity, based on five necrosis-inducing compounds (Minami et al, 2005). Our analysis suggests: A) the biomarker gene set distinguishes severely hepatotoxic treatments from treatments inducing little or no liver damage with good sensitivity and specificity (area under ROC curve ~0.7; representative sensitivity and specificity ~51% and ~88% respectively); B) the biomarker performs less well at distinguishing treatments inducing severe necrosis from treatments inducing other severe injuries; C) the original compounds are returned as positive hits, and the strongest positive hits are statistically enriched for histopathologically-verified necrosis, indicating good reproducibility. The compounds and treatments most correlated and anti-correlated to the biomarker expression pattern could also provide a starting point for generating hypotheses as to the specific molecular mechanisms measured by the biomarker. This example demonstrates the power and utility of biomarker validation in a very large database.

252 USE OF PROTEOMICS-BASED MATHEMATICAL BIODESCRIPTORS IN CHARACTERIZING CHEMICAL TOXICITY.

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In the post-genomic era, "omics" technologies are generating copious data related to the effects of biological and chemical agents on living systems. Proteomics methods such as two-dimensional gel electrophoresis (2-DE) provide data on thousands of proteins and novel methods are needed to extract meaningful information from these proteomics maps. Our research team has developed four methods for characterizing proteomics maps using discrete mathematics and statistics: (1) association of graphs/matrices with proteomics maps, (2) information theoretic biodescriptors, (3) spectrum-like representations of proteomics maps, and (4) statistical approaches to identify critical protein biomarkers. Each of the first three methods generates a single, compact numerical biodescriptor (or a set of numerical descriptors) to characterize the map. The fourth approach identifies a set of critical proteins related to the toxic endpoint being studied. Recently we have shown that these compact biodescriptors and statistically-derived biomarkers can be combined in QSTR (quantitative structure-toxicity relationship) models with traditional chemodescriptors, incorporating information about the state of the biological system into these models. This addition of biological data has been shown to increase the predictive power of toxicity models when estimating the effects of halocarbons on primary rat hepatocytes.

253 COMPUTATIONAL INFORMATICS SYSTEMS FOR SCREENING THE TOXICITY OF NATURAL PRODUCTS.

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Computational toxicology is being proposed for use by regulatory agencies, scientists in the drug discovery process, in the safety assessment of indirect food additives, estimating toxicity of pesticides, and other applied uses. The basic goal is to use advanced in silico (computer-assisted) computational approaches, and the vast experimental chemical toxicity data that exist, to predict various toxicological endpoints on the basis of the classic structure-activity paradigm, probabilistic reasoning, machine learning methods, data mining, and human knowledge. In this study, seven computational toxicology informatics systems were evaluated for their utility in screening for potential chronic toxicity of natural products. Several small organic molecules mainly derived from botanical mixtures were tested in silico, and the results compared to published experimental studies describing their toxic potential. The molecular structures of lipoic acid, epicatechin, 1-octacosanol, estragole, pulegone, and aristolochic acid I were processed through the following computational toxicology software programs. The toxicology software programs evaluated were MDL-QSAR, MC4PC TOXLIT, OncoLogic, Derek, MetaDrug, Vitic, and Leadscope. A consensus model output was generated based upon the data mining and quantitative structure-activity relationship predictive outputs made by these software. Results found good concordance between the in silico consensus modeling with the reported toxicities for each natural product established from experimental toxicity testing. In most cases, the natural products were found to be within the applicability of the domain of the computational toxicology training sets. It is concluded that an external test validation is necessary in order to determine if the consensus model holds with a greater number of compounds, and further evaluation of these toxicological informatics systems could be useful.

254 THE RELATIONSHIP BETWEEN THE AROMATIC RING CLASS CONTENT AND REPEAT-DOSE AND DEVELOPMENTAL TOXICITIES OF PETROLEUM SUBSTANCES BOILING ABOVE APPROXIMATELY 300°F.

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Feuston et al. (1994) found the repeat-dose and developmental toxicities of certain petroleum refinery streams correlate with their polycyclic aromatic compound (PAC) content. Building on this foundation, our work was undertaken within the context of the U.S. EPA HPV Chemical Challenge Program to 1) characterize relationships between PAC content and repeat-dose and developmental toxicities of higher boiling petroleum substances and 2) develop statistical models that could be used to predict critical effects of similar untested substances. We began with 108 dermal toxicity studies and 153 chemical compositional studies on a range of higher boiling petroleum substances. From these we developed sets of matched compositional and effects data for 4 repeat-dose, 4 pre-natal developmental, and 3 post-natal developmental endpoints that included an average of about 80 data points per endpoint. Predictive models were developed for the dose-response relationships between the weight percent concentration of each of 7 separate ring classes of aromatic compounds and specific effects, with high correlations (r= 0.88-0.98) between the observed and model-predicted data. The basic model form was linear, often with variable transformations. The dose group response was the dependent variable with the control group response as a covariate. Non-linear models did not improve model fits. The predictive ability of the models was rigorously tested (validation). The estimated dose-response curves permit the prediction of either the effect at a given dose or the dose that causes a given effect. The models, using compositional data, can be used for predicting the critical toxic effects of certain types of untested petroleum substances.

255 UNCERTAINTY AND VARIABILITY IN PREDICTING PHYSICAL PROPERTIES OF EMERGING MATERIALS AND PHARMACEUTICALS USING COMMERCIAL QSAR MODELS.

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Reliable, up-front information on physical and biological properties of emerging materials is essential before making a decision and investment to synthesize, formulate, scale-up, test, and manufacture a new material for use in both military and civilian applications. Multiple Quantitative Structure-Activity Relationships (QSARs) software tools are available for predicting material's chemical/physical properties and environmental effects. Even though information on emerging materials is often limited, QSAR software output is treated without due uncertainty analysis. We hypothesize that uncertainty and variability in material properties could be too large to provide meaningful results. To test this hypothesis, we predicted Kow for multiple emerging materials. Kow was selected because it is a physical constant and theoretically could be measured with high certainty. It also used as input for calculating biochemical properties of the materials. We have tested uncertainty in QSAR model predictions resulting from potential structural variants in emerging chemical as well as variability across six different commercial Kow calculators (KOWWIN, MarvinSketch, ACD/Labs, CLogP, SPARC). Analysis was done for materials with largely uncertain properties: (i) military compounds (RDX, BTTN, TNT) and pharmaceuticals (Carbamazepine, Bezafibrate, Gemfibrizol). We have also compared QSAR modeling results for well studied materials (PCBs and Triazine herbicides). Our analysis shows that uncertainty due to structural variations of the emerging chemicals may be several orders of magnitude. The variability across six software packages is very high (10 orders of magnitude) for emerging materials while it is low for traditional chemicals (e.g., PCBs and triazine herbicides). We conclude thus that the use of QSAR model for emerging materials screening requires extensive model validation and coupling QSAR output with available empirical data and other relevant information.

256 NOVEL CHIRALITY INDICES TO MODEL BIOACTIVITY/TOXICITY OF DIASTEREOMERS.

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If an organic compound has one chiral carbon (stereogenic center), then it has two configurations. Thus 2^n stereoisomers are possible for a compound with n chiral centers, and the 2^n stereoisomers will exist as 2^(n-1) pairs of enantiomers, and each pair of enantiomers is diastereomeric with any other pair. Enantiomers have identical physicochemical properties but differ in their biological, pharmacological and toxicological properties due to the stereo-specificity of the biological receptors. Hence, computational chemists are faced with the challenge to develop models that predict the pharmacological, toxicological and biological activities of diastereomers. Conventional QSAR modeling using simple computed molecular descriptors or physicochemical properties fails in handling compounds that exhibit polychiral diastereomerism. We recently developed a novel method for the numerical characterization of chirality. The new chirality indices are called relative chirality indices (RCI) because they not only differentiate the diastereomers but also order them on different scales chosen to describe the ligands attached to the chiral center(s). The new indices were found to correctly order the repellency of diastereomeric mosquito repellents. Application of the new chirality indices in predicting toxicity of diastereomeric compounds will also be presented.

257 AN *IN SILICO* APPROACH TO ASSESSING RISK OF 1-BROMO-2-CHLOROETHANE.

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Thousands of chemicals may be found at environmental waste sites whose potential adverse human health effects are not easily identifiable, typically due to a paucity of available toxicity data. Integration of toxicologically uncharacterized compounds, such as 1-bromo-2-chloroethane (BCE), into estimations of human health risk due to potential environmental exposures is ideal and may be facilitated through the use of alternative predictive tools such as Quantitative Structure Activity Relationship (QSAR). The available toxicity database for BCE is extremely limited; there are no known animal bioassay data available for assessment of risk by the oral or inhalation exposure routes. Several in vitro studies have reported BCE-induced genotoxicity (e.g. mutagenicity), and the relative activity appears to be comparable to the structurally related chemicals 1,2-dibromoethane (1,2-DBE) and 1,2-dichloroethane (1,2-DCE), both of which have been shown to induce cancer in rodents. Although there are no known in vivo studies to suggest carcinogenicity for BCE, it was hypothesized that a QSAR modeling approach would provide a useful quantitative prediction of cancer risk via the oral route of exposure. A QSAR model for predicting carcinogenic potency (oral slope factor; OSF) using physicochemical properties of 70 chemicals from the U.S. EPA's Integrated Risk Information System (IRIS) was developed and then applied to BCE. The QSAR model was generated using stepwise multiple linear regression (MLR) with high correlation (r2=0.771) and predictability (q2=0.732). The predicted OSF for BCE is 2.31 (mg/kg-day)-1 which is significantly greater than the OSF of 9E-2 (mg/kg-day)-1 for 1,2-DCE, but very close to the OSF of 2.0 (mg/kg-day)-1 for 1,2-DBE, suggesting a cancer potency similar to this known carcinogen. This modeling exercise demonstrates the utility of QSAR in predicting potential human health risk posed by chemicals with limited data.

258 AN INTELLIGENCE NETWORK FOR DRUG-INDUCED LIVER INJURY.

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Drug-induced liver injury (DILI) is the leading cause of acute liver failure and the most common reason for drug development failures. DILI is frequently idiosyncratic, occurs in low incidence and in most cases is mechanistically poorly understood. Information relevant to DILI is distributed across disparate and diverse databases, hindering access to a comprehensive source of knowledge on liver injury. The ability to seamlessly harness historic public domain knowledge on compounds that perturb liver function would provide insights into complex pathophysiological mechanisms and improve our ability to develop new drugs with reduced potential for DILI. We have developed an Intelligence Network for DILI. Using extensive vocabularies with lexical and linguistic text-mining tools, assertions in the form "Compound A causes/affects Liver Observation B" were generated from public domain data sources (including literature, toxicity and regulatory sources). Compound vocabularies spanned over 150,000 developmental, marketed, and withdrawn drugs, environmental and test chemicals. Vocabularies for liver observations included pathological and physiological changes at the organ, cellular and sub-cellular levels. Incorporation of chemical structure and modelling methods provided key observations on structure-activity relationships of hepatic injury. Systematic analysis of initial data identified 120 compounds with >300 liver-associated pathologies across humans, preclinical species and in vitro models. There were effects on more than 660 subtypes of liver physiology, including apoptosis, bile flow, and hepatocyte proliferation, which provided rich and valuable information on mechanistic hypotheses underlying the phenotypic manifestations. This assess-

ment demonstrates that the Intelligence Network methodology is a robust and promising new approach to exploiting multiple and disparate data sources, which can be extended to other areas of major organ injury.

259 THE GENETIC AND ENVIRONMENTAL PATHWAYS TO COMPLEX DISEASES.

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The pathogenesis of complex diseases involves the integration of genetic and environmental factors over time, making it particularly difficult to tease apart relationships between phenotype, genotype, and environmental factors using traditional experimental approaches. Rapid progress in molecular techniques has resulted in an impressive collection of data linking both phenotypes and environmental factors to particular genes. Using gene-centered databases to define linkages between phenotypes or environmental exposures and genes, we have developed networks of complex diseases and environmental factors, using evolutionarily conserved molecular pathways as the unifying system to define relationships. Illustrating the utility of our analysis, we have determined PPAR and retinoic acid signaling pathways are common to genetic and environmental factors for metabolic syndrome phenotypes. The higher order structure generated through development of a pathway interconnectivity network shows signal transduction and hormonal pathways versus highly conserved metabolic pathways are more often associated with both genetic and environmental factors. Most importantly, the integration of genetic association analyses on human phenotypes with mechanistic action of exogenous substances offers a biological construct linking putative genetic and environmental components across numerous complex diseases, providing specific hypotheses useful for prioritizing future research in complex diseases.

260 IDENTIFICATION OF POSITIONALLY CONSERVED DIOXIN RESPONSE ELEMENTS IN ORTHOLOGOUS HUMAN, MOUSE, AND RAT GENES.

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Computational identification and prediction of dioxin response elements (DREs) within gene regulatory regions is one method used to identify novel, putative aryl hydrocarbon receptor (Ahr) binding locations. This aids the phenotypic anchoring of toxicogenomic data to the AhR-signaling mechanism, and can facilitate cross-species comparisons as well as comprehensive microarray data interpretation. However, the utility of computationally identified putative DREs is limited due to the high false positive rate of most detection methods, and the frequent occurrence of the DRE core element (GCGTG) in the genome. The identification of positionally conserved DREs (the core plus flanking sequences) within orthologous human, mouse, and rat genes partially adresses this limitation by increasing the probability of identifying functional DREs. We use a position weight matrix (PWM) to identify putative DREs within regulatory regions (-10,000bp relative to the transcription start site through the 5'-UTR) of species-conserved and species-specific dioxin-responsive genes. Agglomerative hierarchical clustering of the DRE sequences across the human, mouse, and rat is used to identify conserved DREs. In addition, an empirical distance threshold decreases the false positive clustering rate, and a map of the DREs of all three orthologous genes illustrates the positionally conserved orthologous DREs. Maps for species-conserved dioxin responsive genes, such as CYP1A1, CYP1B1, and Aldh3a1 identify the location of positionally conserved DREs in the regulatory regions. This approach is being expanded to prioritize species-specific dioxin-responsive genes that warrant further validation. The combination of this method with other response elements may facilitate the elucidation of the regulatory grammar required for AhR-mediated gene regulation. Funded by NIEHS Superfund grant P42 ES04911.

261 VALIDATING ALERTS FOR THE PREDICTION OF MUTAGENICITY.

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The growing use of in silico methods for the prediction of toxicity has led to increased scrutiny of their predictive performance. In the context of the OECD Principles for (Q)SAR Validation, introduced to facilitate the consideration of such methods for regulatory purposes, this is reflected in the principle advising of the importance of "appropriate measures of goodness-of-fit, robustness and predictivity". Derek for Windows is a knowledge-based expert system designed to predict the toxicity of a chemical from its structure. The knowledge base is composed of alerts, example compounds and rules each of which contributes to the predictions made by the system. The alerts define chemical environments associated with toxicity and are supported by a summary of the evidence used to derive them and corresponding literature citations. Currently, the Derek for Windows knowledge base includes eighty-five mutagenicity alerts for the prediction of Ames test activity.

Recently, a new feature has been introduced into the Derek for Windows program which allows validation data for each alert to be displayed when it is activated by a query compound. This validation display has been populated for Ames test mutagenicity alerts by assessing their predictive performance for four data sets, three of which include Ames test data from the public domain whilst the remaining data set makes use of proprietary Ames test data arising from a data sharing initiative.

In addition to assisting the Derek for Windows user in understanding the significance of the presence of a given alert, the performance metrics also allow knowledge base development efforts to be directed to those alerts which are performing least effectively.

Future plans for the alert validation feature include the introduction of results from additional analyses of Ames test data, including for proprietary data sets held in-house by Derek for Windows member organisations, and extension of the feature to alerts relating to the prediction of other toxicological endpoints.

262 ANALYSIS OF TRANSCRIPTION FACTOR BINDING MOTIFS IN UPSTREAM REGIONS OF CO-REGULATED GENES: A METHOD FOR CREATING REGULATORY NETWORKS.

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Establishing a link between regulatory mechanism and transcriptional response is key to extracting biological meaning from global gene expression experiments. We have established a method for analysis of microarray data that interrogates the promoter regions of groups of co-regulated genes to determine over-representation of transcription factor binding sites. We created a transcription factor binding site (TFBS) tool that uses a pre-computed database containing TFBSs for thousands of genes to statistically determine over-representation of binding sites within subsets of genes. Twenty-nine genes known to share common regulatory control from the NF-κB transcription factor were used to validate the TFBS tool, and the result of this validation showed that expected NF-κB TFBS were successfully identified. For additional validation, we used a set of known CREB-regulated genes, resulting in expected identification of CREB TFBS over-representation by our tool. We then effectively applied the TFBS tool to analysis of transcript profiles derived from microarrays of RNA extracted from various tissues after treatment with protein kinase inhibitors H89 (N-(2-[p-Bromocinnamylamino-]ethyl)-5-isoquinolinesulfonamide) and LSN2109761. Our results show that transcriptional activities could be connected to pathways that are modulated by the kinase targets of these inhibitors. Furthermore, several pathways not specifically targeted by the compounds appeared to be connected to the transcripts observed. These results support the expanded potential for TFBS analysis to reveal on and off-target activities for small molecules in vivo.

263 THE USE OF GENE EXPRESSION DATA FROM MULTIPLE EXPOSURE TIMES FOR THE DEVELOPMENT OF GENOMIC BIOMARKERS OF CARCINOGENIC POTENTIAL.

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Two year rodent cancer bioassays are currently the standard by which the carcinogenic potential of chemicals are judged because chemical induced tumor development (cancer) is the end point of these studies. With the advent of transcriptional profiling using microarrays there has been a concerted effort to identify biomarkers capable of predicting carcinogenic potential based on gene expression data collected after short term chemical exposure. While it would be clearly advantageous to be able to use chemical exposures of short duration, there has been little effort to determine what exposure time(s) may yield the most informative information for predictability. We exposed male F344 rats to 4 male rat hepatocarcinogens (aflatoxin B1, N-nitrosodimethylamine, 1-amino-2,4-dibromoanthraquinone, and methyeugenol) and 3 non-carcinogens (acetaminophen, tryptophan, ascorbic acid) and collected hepatic gene expression data after chemical exposures of 3, 15, and 90 days. An additional group of animals exposed to each carcinogen or non-carcinogen for 90 days was then held without further chemical exposure for an additional 60

days. Using a nearest centroid classification algorithm we found the ability to correctly classify a carcinogen or non-carcinogen was greatly improved using data from multiple exposure times compared to data from a single exposure time.

264 STRUCTURAL REQUIREMENTS FOR ORGANOPHOSPHORUS INSECTICIDES TO INHIBIT *IN VIVO* KYNURENINE FORMAMIDASE IN CHICKEN EMBRYOS AND MICE.

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Kynurenine formamidase [EC 3.1.5.9] is a proposed target in chicken teratogenesis [Seifert, J. and Casida, J.E.C. (1981) Progress in Pest. Biochem. 1, 219-246] and alteration of L-tryptophan metabolism in mice [Pewnim, T. and Seifert, J. (1993) Eur. J. Pharmacol. 248, 237-241] caused by organophosphorus insecticides (OPIs). Pyrimidinol phosphorothionates and crotonamide phosphates were the most potent toxicants in both chicken embryos and mice. Our current investigation focused on OPI structural features that determine their in vivo potency to inhibit kynurenine formamidase in mice liver and chicken embryo yolk sac membranes. 3D QSAR of the series of OPIs obtained by using COMFA showed a strong correlation between the electrostatic and steric fields and OPI in vivo potency to inhibit kynurenine formamidase in both animal species. An increased positive electrostatic charge in OPI leaving groups such as disubstituted pyrimidinol and/or N, N-dimethyl methylcrotonamide moieties in diazinon and dicrotophos, respectively, enhances OPI inhibitory potency. Bulkiness of 4, 6-substituents in pyrimidinol phosphorothioates and/or alkyls in N, N-dialkyl alkylcrotonamide phosphates favors the enzyme inhibition. The electrostatic fields formed around the phosphoryl and/or thiophosphoryl groups that support in vivo inhibition of kynurenine formamidase are distinct in mice and chicken embryos. Validity of the models based on 3D QSAR was supported by a good agreement of the computer-predicted data calculated for the series of structural analogs of the best kynurenine formamidase inhibitors, diazinon and dicrotophos, with their toxicity data.
Acknowlegement. The author thanks to Dr. W.H. Welch of the University of Nevada in Reno for allowing me to use the UNR Molecular Modeling Center and for his valuable advice and assistance in the molecular modeling part of this project.

265 PURIFICATION AND CHARACTERIZATION OF RECOMBINANT HUMAN PARAOXONASE FROM *ESCHERICHIA COLI.*

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Paraoxonase (PON1) is a liver and plasma HDL-associated enzyme which hydrolyzes specific organophosphorus (OP) compounds and is the leading candidate to be engineered for protecting against nerve agent exposure. Stoichiometric scavengers such as butyrylcholinesterase have been tested for protecting against OP exposure; however, catalytic scavengers such as PON1 will require dramatically lower quantities of protein for therapeutic applications. Human PON1 has been shown in mouse models to protect against OP exposures including chlorpyrifos oxon and diazoxon. Improved catalytic efficiency is required for use in protecting against nerve agent exposure. We have developed a protocol for engineering and expressing active, untagged recombinant human PON1 (rhPON1) in *E. coli*. Purification of PON1 enzyme to near homogeneity has been accomplished using a series of column chromatography methods involving both anion exchange and hydrophobic interaction chromatography. The purified rhPON1 is non-toxic when injected into mice and has a half-life similar to purified human plasma PON1. Supported by NIEHS ES09883.

266 IDENTIFICATION OF CYTOCHROME P450 SUBFAMILIES INVOLVED IN ORGANOPHOSPHOROUS PESTICIDES BIOTRANSFORMATION IN MALE REPRODUCTIVE TRACT OF MICE.

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Organophosphorous pesticides (OP) are biotransformed by different cytochrome P450 (CYP) isoforms to form the active metabolites, the oxons, which are considered more toxic. Methyl parathion (Me-Pa), an OP widely used in agriculture, is associated with male reproductive effects, including poor semen quality and sperm chromatin alterations. Me-Pa is activated in human liver by CYP1A2, CYP2B6, 2C19 and CYP3A4 and it has been suggested that OP have to be metabolized in situ to exert their toxic effects. Therefore, our objective was to identify the presence and activity of CYP1a2 isoform, and CYP2b, 2c and 3a subfamilies in mice reproductive tract (testes and epididymis-vas deferens). Two groups of CD1 adult male mice: control and phenobarbital (PB)-treated (80 mg/kg/day for 3 days) were sacrificed, and liver, testes and epididymis-vas deferens were extracted and processed to obtain microsomes. CYP isoforms were identified by Western blot analysis using anti-rat or anti-human CYP antibodies and by O-dealkylation of methoxy- (MROD; CYP1a2) and pentoxy-resorufin (PROD; CYP2b) and by 6β- (CYP3a) and 2α- and 16α-testosterone hydroxylation (-OHT; CYP2c). In control animals, the four isoforms were immunodetected in liver and testes, and only CYP2c and 3a in epididymis-vas deferens. While in PB-induced animals, the four isoforms were detected in all tissues. MROD and PROD activities and 16α- and 6β-OHT were also observed in all tissues. PB induced some of the isoforms activity, being higher in liver in all cases. These data indicate that the main CYP isoforms involved in Me-Pa activation in liver are present in testes and epididymis-vas deferens, suggesting that Me-Pa metabolism in reproductive tissues may be involved in its toxicity. (Project supported by Conacyt-Grant #58213).

267 CYP-SPECIFIC PBPK/PD MODELS FOR ASSESS HUMAN RISK TO ORGANOPHOSPHOROUS PESTICIDES.

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Recently, recombinant human CYPs were utilized to quantify the CYP–specific kinetic variables (Km, Vmax and Vmax/Km, or intrinsic clearance (Clint)) for the rate of desulfation (activation) and dearylation (detoxification) of chlorpyrifos and parathion, two model organophosphate pesticides (OPs), for future use in refining human PBPK/PD models (Foxenberg et al., 2007). CYP2B6 and CYP2C19 exhibit low Km and high Vmax values (high Clint) for the desulfation and dearylation of chlorpyrifos, respectively, supporting their role as the primary enzymes that regulate the respective activation and detoxification reactions at low-level, real-world human exposures. In the case of parathion, Clint values for desulfation and dearylation reactions with these enzymes were also high, but similar, with CYP2B6 and CYP2C19 mediating both reactions. The CYP-specific PBPK/PD model incorporates enzyme specific parameters (Km, Vmax, and CYP content) to investigate how variability in CYP content and activity alters predicted cholinesterase inhibition. Utilizing the CYP-specific PBPK/PD model, a single dose of chlorpyrifos was predicted to produce greater than a 2-fold difference in butyrylcholinesterase (BuChE) inhibition between simulations that varied CYP2B6 and 2C19 content by a factor of 4. In contrast, a single exposure to parathion was predicted to produce a similar level of BuChE inhibition in simulations that varied CYP2B6 and 2C19 content by a factor of 4. This demonstrates that the output from the CYP-specific model can be affected by enzyme content and pesticide specific kinetic parameters. Since human hepatic content of specific CYPs exhibits marked population variability, the building of models that use kinetic parameters for OP metabolism by specific human CYPs and the hepatic content of specific CYPs should prove to be more accurate and more easily modified to address factors such as age and polymorphisms which may affect CYP protein expression and activity. (Supported by U.S. EPA STAR Grants R-830683 and R-833454)

268 EVALUATION OF A WEIGHTED TEST IN THE ANALYSIS OF ORDINAL GAIT SCORES IN AN ADDITIVITY MODEL FOR FIVE OP PESTICIDES.

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Appropriate statistical analyses are critical for evaluating interactions of mixtures with a common mode of action, as is often the case for cumulative risk assessments. Our objective is to develop analyses for use when a response variable is ordinal, and to test for interaction in a mixture of chemicals. The typical cumulative logit model for ordinal data assumes proportional odds; when that assumption is violated, a continuation-ratio logit (CRL) model may be used. A CRL additivity model was developed and may be compared to mixture points of interest to test for interaction. The study exposed mice to an environmentally-relevant mixture of five organophosphate pesticides with Gait Score ranked in four categories: (1) no, (2) mild, (3) moderate, (4) severe abnormalities. For these data the proportional odds assumption was violated and a CRL model was used.
With the CRL model, the multinomial distribution is factored into independent conditional binomial analyses so that standard software for binary data can be used. Generally, as the ordinal categories progress, the sample size in each analysis decreases. In an unweighted analysis, each independent statistic has equal weight although the sample sizes may not be equal. A weighted statistic was developed with more weight given to analyses with higher sample sizes and less weight to analyses with fewer observations.

Both the weighted and unweighted analyses of the data revealed synergy at both a low and moderate dose. A simulation study based on these data was performed to evaluate the power of the weighting scheme with varying degrees of interaction. The power of the test depends on how extreme the probability distribution is for additivity, which is dose-dependent. The weighted statistic had greater power partly due to the decrease in the weighted degrees-of-freedom for the test. (Partially supported by NIEHS #T32ES007334 and does not reflect USEPA policy.)

269 CUMULATIVE RISK ESTIMATION USING EXPOSURE AND DOSE MODELS TO THREE N-METHYL CARBAMATES: CARBARYL, ALDICARB, AND CARBOFURAN.

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The cumulative risk resulting from the aggregate exposure to three N-methyl carbamate insecticides – carbaryl, aldicarb, and carbofuran – was investigated for a sample population studied in the Stochastic Human Exposure Dose Simulation (SHEDS) model. The SHEDS model simulates oral dietary exposure patterns along with exposure to aldicarb from drinking water. These exposure scenarios were then used as inputs to a cumulative human physiologically-based pharmacokinetic/pharmacodynamic (PBPK/PD) model developed in the Exposure Related Dose Estimating Model (ERDEM) system. Distributions of acetylcholinesterase (AChE) activities in the red blood cells and brain, and urine biomarker concentrations were evaluated for different exposure scenarios. Investigations included the comparison of consecutive multi-day vs. single day SHEDS scenarios, effects simulated by the PBPK/PD model to an effective exposure half-life model, and impacts of individual chemicals and routes. The population distributions of the above toxicological endpoints were assessed to evaluate the cumulative risks caused by the aggregate exposure of the three chemicals. The methodology used in this study demonstrates a plausible method for in silico human population risk assessment.

Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy.

270 REPEATED DOSE TWENTY-ONE DAY DERMAL TOXICITY STUDY WITH CARBOFURAN, A CARBAMATE INSECTICIDE.

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Carbofuran (CF), was administered dermally to the shaved backs of ten Crl:CD®(SD) rats/sex per group, six hours per day for 21 days at 0 (deionized water), 15, 25, 50 and 250 mg/kg body weight/day. A gauze dressing secured with tape and Vetrap® held the CF in place and collars were worn to prevent ingestion. At the end of each exposure period, the CF was rinsed from the backs with deionized water and 1% solution of baby shampoo. A functional observational battery (FOB) followed by motor activity evaluations were conducted on DS (day of study) 20, prior to CF administration. Within one hour of the end of the exposure period on DS21, whole blood samples were collected for red blood cell (RBC) cholinesterase (ChE), hematology, and clinical chemistry assays and the brain was excised for ChE assays. In males, no adverse clinical observations related to the test substance occurred. Three females in the 250 mg/kg/d group had tremors and two had fasciculations and excess salivation. Mean body weights and body weight gains in males did not differ significantly among groups. In females, significantly reduced body weight gains for DSs 1 to 21 and body weight on DS 21 occurred in the 250 mg/kg/d group compared to controls. There were no effects on skin reactions, feed consumption, ophthalmology, FOB, motor activity, necropsy findings, hematology, clinical chemistry or histopathology in either sex. There were no significant effects on terminal body weights, absolute organ weights and the ratio of organ weights to the terminal body weight for males. The terminal body weight and the absolute liver weight in females were significantly reduced at 250 mg/kg/d. No statistically significant reductions in RBC ChE values occurred. A statistically significant reduction in brain ChE values occurred only for the female rats at 250 mg/kg/d. The no-observable-adverse effect level was 50 mg/kg/day and the LOAEL was 250 mg/kg based upon effects in females. This value provides an accurate value for use in calculation of worker risk from exposure to carbofuran.

271 AN EMPIRICAL APPROACH TO SUFFICIENT SIMILARITY IN DOSE-RESPONSIVENESS IN A MIXTURE OF 11 PYRETHROIDS.

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This research evaluated sufficient similarity in dose-responsiveness using a mixture of 11 commonly used pyrethroids (PYR; Wolansky et al, 2005). When toxicity data are not available for a chemical mixture of concern, the U.S. EPA guidelines allow use of reference mixtures considered sufficiently similar in terms of chemical composition and component proportions. A supplementary approach, using statistical equivalence testing logic and mixed model theory has been developed to define sufficient similarity in dose-response for chemical mixtures containing the same chemicals with different ratios. This methodology requires all chemicals to be present in both the reference and candidate mixture. An extension to this method is demonstrated that allows for the absence of chemicals in the candidate mixture based on a measure of sufficient similarity using Euclidean distance. Adult male LE rats were acutely exposed to a mixture of 11 PYRs (5 Type I, 5 Type II, and 1 mixed Type I/II PYRs). Dose-response functions for effects on motor activity were developed using 1 hr figure-8 maze sessions. Relative potencies were calculated from these data and formed the mixing ratio for the reference mixture. The mixing ratio for exposure to these 11 PYRs was assumed to be a random process and the current work estimated how often a resulting candidate mixture would be sufficiently similar in dose-responsiveness to the reference mixture. A simulation study with 100 candidate mixtures of the same 11 PYRs was conducted with assumed exposure distributions for each chemical ratio and 81% of the candidate mixtures were determined to be sufficiently similar in dose-responsiveness to the reference mixture. This approach evaluates sufficient similarity in the mixing ratio for observed data and an unobserved random process for the mixture. (Partially supported by NIEHS #T32ES007334 and does not reflect USEPA policy. These data/research are not associated with Monsanto).

272 RATIONALE FOR THE USE OF THE CIS:TRANS ISOMER RATIO OR TOTAL ISOMER CONTENT OF CYPERMETHRIN, ZETA CYPERMETHRIN, AND PERMETHRIN FOR HUMAN HEALTH RISK ASSESSMENT.

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Synthetic pyrethroids show high stereospecificity for insecticidal activity and mammalian toxicity. Permethrin (PER) and cypermethrin (CYP) have 4 and 8 stereoisomers, respectively, differing only by the α-carbon cyano group in CYP. The different ratios of cis:trans isomers of PER affect its acute oral toxicity. Cis:trans PER ratios of 80:20, 60:40, 50:50, 40:60, 30:70 and 20:80 produced rat oral LD50 values of 224, 445, 1000, 1260, 1684 and 6000 mg/kg, respectively, dosed in corn oil. The cis isomers contribute significantly more to the acute toxicity of PER than the trans isomers. Mammalian toxicology studies for registration are conducted on the technical active ingredient (TAI). Humans are exposed to the mixture of isomers comprising the TAI; therefore, it is appropriate to use the TAI isomer mix for risk assessment when both the hazard and exposure are based on the same or similar mixtures. Residues of zeta–cypermethrin (ZCYP) on corn were characterized as cis:trans and R:S at the α-cyano center in 31 day forage and 80 day stover samples and compared to the ratios of isomers in the TAI applied to the plant. The ratios of the residues of cis:trans isomers of ZCYP, and the R:S ratio in ZCYP on the corn plant were unchanged compared to those of the TAI applied to the plant. In an apple metabolism study, 100% cis-CYP was partially converted to trans-CYP (30% in leaf, 15% in peel), reducing the mammalian toxicity of the residues, relative to the TAI. Trans-CYP was metabolized without conversion to cis-CYP isomers. The metabolism of CYP / ZCYP by crops results in the same cis:trans isomer mix or one that is reduced in acute mammalian toxicity (less cis isomer). In conclusion, the use of the cis:trans ratio or the total isomer content of the TAI is appropriate for human risk assessment for these pyrethroids because hazard is based on the TAI and crop residues are similar or less toxic than the TAI.

273 ENANTIOSELECTIVE ESTROGENIC ACTIVITY OF PYRETHROID INSECTICIDES.

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The growing list of emerging environmental contaminants includes potential endocrine disrupting chemicals, some of which are chiral agrochemicals consisting of optical isomers called enantiomers. Among the modern-use pesticides, chirality exists in all pyrethroid insecticides. Pyrethroids and their metabolites have been reported to bind to estrogen receptors in the yeast estrogen screen. Enantioselective

uptake in fish, as well as enantioselective estrogenic potential in the E-SCREEN assay was also recently reported for cis-bifenthrin. These results suggest that the overall risk of pyrethroids may depend on the fate and effects of the active enantiomers rather than the racemate. In the present study we investigated the occurrence of enantioselectivity in the estrogenic activity of the pyrethroids, permethrin and cypermethrin, in fish. Chiral selective HPLC technique was used to resolve and isolate pyrethroid enantiomers for the evaluation of estrogenic activity. Preliminary results indicate mild vitellogenin induction in adult male Japanese medaka (Oryzias latipes) following 7 days exposure to permethrin, with the 1S-cis and the 1S-trans enantiomers eliciting up to 3 times greater response than the corresponding 1R-cis and 1R-trans enantiomers, respectively. Similarly, for cypermethrin the 1S enantiomers, although observed to be less acutely toxic than the corresponding 1R enantiomers, induced vitellogenin in adult male Japanese medaka up to 5 times higher than the controls. Preliminary in vitro evaluation using primary rainbow trout (Oncorhynchus mykiss) hepatocytes indicated induction of vitellogenin-mRNA by cypermethrin enantiomers at levels significantly higher ($p \leq 0.05$) than the controls. Stereoselectivity was also observed in this effect. The enantioselective effects of these modern-use pesticides could be important considerations in future risk assessment and regulatory decisions with regards to chiral agrochemicals.

274 ESFENVALERATE ACTS AT THE HYPOTHALAMUS TO SUPPRESS THE AFTERNOON RISE OF LUTEINIZING HORMONE IN PREPUBERTAL FEMALE RATS.

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Pesticides are used worldwide to control agricultural and household pests. According to the Environmental Protection Agency, in 2001 over 78 million households in the United States used pesticides. One of the most frequently used classes is the synthetic pyrethroids. While less acutely toxic to humans than organochlorines or organophosphates, in vitro studies have suggested pyrethroids may be estrogenic, leading them to be listed as potential endocrine disrupting agents. We assessed pubertal effects by administering low doses (1.0 and 5.0 mg/kg) of the type II pyrethroid, esfenvalerate (ESF), by oral gavage to female SD rats. We found that ESF suppressed serum estradiol and delayed pubertal onset, indicating that it was not acting as an estrogen in vivo. To assess if esfenvalerate was exerting an effect at the hypothalamic and/or pituitary level, we performed the following experiments. Female Sprague Dawley rats were gavaged once daily from postnatal day 22 until 29 with ESF at 1.0 mg/kg or corn oil. Rats were anesthetized and cannulae inserted into the right external jugular vein. On day 30, three basal blood samples (200 µl) were drawn at 15-minute intervals from each rat at 1000 hr, and again at 1500 hr. After the third afternoon sample, all animals received an IV injection of luteinizing hormone releasing hormone (LHRH; 25 ng/rat) then two more samples were drawn. A second experiment was performed as stated except animals received N-Methyl-DL-Aspartic Acid (NMA; 40 mg/kg) instead of LHRH. In both experiments controls exhibited a rise in basal afternoon luteinizing hormone (LH) as compared to morning levels ($P<0.05$) while pesticide treated animals did not. Additionally, LHRH stimulated release of LH was the same for pesticide treated and control animals indicating the pituitary was not affected. While LH levels in the control animals significantly increased following NMA injection ($P<0.05$), ESF treated animals showed an even higher level of LH secretion indicating a hypersensitivity to NMA and suggesting a hypothalamic effect.

275 PYRETHROID EXPOSURE OF ZEBRAFISH (DANIO RERIO) EMBRYOS RESULTS IN ABNORMAL DEVELOPMENT.

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Pyrethroid pesticides are used in both household and agriculture applications. Because of their relatively low acute mammalian toxicity, they are often thought of as "safe" alternatives to other classes of pesticides. However, there is increasing concern that developing organisms are more sensitive to the adverse effects of pesticides. Here, we sought to determine whether early exposure to the two groups of pyrethroid pesticides (Type I and II) would have a long-term impact on development using zebrafish as a model organism. Developing zebrafish were exposed to permethrin (Type I), cypermethrin (Type II), or deltamethrin (Type II) at concentrations ranging from 1 to 100ng/mL. Exposure to all of the pyrethroids results in adverse effects on the developing zebrafish. Deltamethrin and cypermethrin were more potent than the permethrin, with exposure at the highest dose resulting in 70-73% mortality. No significant increase in mortality was observed following permethrin exposure up to 100 ng/mL. At sub-lethal concentrations, pyrethroid exposure caused a variety of developmental lesions, including pericardial and yolk sac edema, spinal curvature, and craniofacial abnormalities. Embryos exposed to the deltamethrin and cypermethrin demonstrated all the described lesions; however, permethrin exposed embryos only had increased pericardial and yolk sac edema. Exposure to each of the pyretrhoids also resulted in increased activity and uncontrolled neuromuscular twitching, which was more evident in exposure to class II pyrethroids. These data demonstrate that embryonic exposure to pyrethroid pesticides results in abnormal development and indicates that Type II pyrethroids may be more potent than Type I. These studies also suggests that the zebrafish is an ideal model to examine the effects of pesticide exposure on developing vertebrates. Supported by NIEHS Grants R01ES015991 and P30ES005022

276 EVALUATION OF VASCULAR TOXICITY OF PERMETHRIN IN HUMAN ENDOTHELIAL CELL AND MOUSE ES CELL.

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Recently, angiogenesis inhibitors have been researched with great interests in medical treatment of tumors or cerebrovascular disease since vascular is essential for functions of all organs of animals, and many chemicals were evidenced their angiogenesis inhibitory effects. Currently, a fear has been raised that some of environmental chemicals may be angiogenesis inhibitors. We evaluated the effects on angiogenesis of permethrin, a widely used insecticide with tubular formation assay and differentiation systems of mouse ES cells in the present study.
In tubular formation assay using human brain endothelial cells, treatment with permethrin reduced the length between branches and increased not-connected branches. Same changes were detected in the endothelial cells treated with thalidomide, a well established angiogenesis inhibitor. The results suggest permethrin acts as an angiogenesis inhibitor.
To evaluate effects of permethrin on differentiation of endothelial cells, embryoid bodies (EBs) were formed from mouse ES cells in VEGF containing medium added permethrin or thalidomide. Then, they were cultured in type I collagen gel without compounds. Although, after over night culture in gel, no noticeable change was observed in EBs treated with DMSO as vehicle, mesoderm like cells were migrated from 35-40% of EBs treated with permethrin or thalidomide. Vascular-like sprouts were furthermore observed in about 60% of DMSO treated EBs after 2-day culture in gel. However, 30% and 10% sprouted of permethrin-treated and thalidomide-treated EBs, respectively. These results indicated that permethrin affected on differentiation of mesoderm and inhibited differentiation of endothelial cells from ES cells as well as thalidomide.
In conclusion, the present study revealed a new toxic potential for permethrin in angiogenesis. Permethrin as well as thalidomide may be an angiogenesis inhibitor in environment.

277 EVIDENCE FOR LACK OF GENOTOXICITY OF ZETA-CYPERMETHRIN.

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Zeta-cypermethrin, a pyrethroid insecticide, was evaluated by five genotoxicity tests: the Ames test, CHO/HGPRT forward gene mutation assay, chromosomal aberrations test in CHO cells, unscheduled DNA synthesis (UDS) in primary rat hepatocytes, and the chromosomal aberrations test in rat bone marrow in vivo. In the Ames test, negative results were observed in strains TA 1535, TA 1537, TA 1538 and TA 98 with and without metabolic activation system and in strain TA 100 with activation. Without metabolic activation, a slight increase in mutation rate was seen in test strain TA 100 at 3333 and 10000 µg/plate with a maximum two-fold increase in mean revertants/plate at 10000 µg/plate. Therefore, zeta-cypermethrin was weakly positive in one strain, TA100, without metabolic activation, and negative in the other strains. Zeta-cypermethrin did not induce mutation at the HGPRT locus in Chinese Hamster Ovary (CHO) cells with or without metabolic activation system in two separate assays each with confirmation. Among the battery of genotoxicity assays to screen for possible mutagens, CHO/HGPRT and Ames tests detect gene mutations induced by chemicals in mammalian cells and bacteria, respectively. Compared to the Ames test, the CHO/HGPRT assay is more relevant to human exposure because it is conducted in mammalian cells. Zeta-cypermethrin did not induce chromosomal aberrations in CHO cells with or without metabolic activation system in vitro and in rat bone marrow in vivo. Zeta-cypermethrin did not cause a significant increase in unscheduled DNA synthesis in primary rat hepatocytes. Based on the weight of evidence, it is concluded that zeta-cypermethrin is not genotoxic.

278 EFFECTS OF ATRAZINE ON BLOOD PHYSIOLOGY IN ADULT FEMALE WISTAR RATS.

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Atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine) is one of the most commonly used herbicides in the United States. Exposures of rodents to ATRA have result in the suppression of luteinizing hormone surge. Previously, we have reported that diaminochlorotriazine (DACT), an atrazine metabolite, forms a covalent adduct with rat hemoglobin at Cys-125, an residue not found in human hemoglobin. In the present study, we investigated the possible physiological effects of the presence of DACT adducts on hemoglobin. Previous work from our lab demonstrated that the level of DACT/hemoglobin adduct formation peaks at 72 hours and is sustained for at least 10 days. In the present study, ovariectomized Wistar rats were administered atrazine (200mg/kg) or vehicle by gavage and seventy-two hours later, blood samples were taken and analyzed using an Abbott Precision 1-STAT CG8+ blood analyzer. Results showed no significant differences in blood levels of glucose, sodium, potassium, calcium, hemocrit, hemoglobin, pH, PO2, or sO2 levels between treatment groups. However, Total CO2 (29.33±.667 vs. 33.25±.366 mmol), PCO2 (55.58±1.65 vs 62.45±1.53 mmol), HCO3 (27.53± .605 vs 31.43±.910 mmol/L), BEecf (1.18± .703 vs. 5.13± .35 mmol/L) were all elevated in atrazine treated animals compared to vehicle treated controls. These results indicate that the DACT adduct formation with rodent hemoglobin Cys-125 does not reduce O2 carrying capacity of blood but apparently modifies the CO2 concentration in blood in atrazine-treated animals. The physiological impact of these changes to the rodents remains unclear.

279 REVERSAL OF ATRAZINE DEPENDENT EFFECTS ON GNRH NEUROENDOCRINE FUNCTION IN WISTAR RATS.

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Our lab and others have shown that the preovulatory luteinizing hormone (LH) surge is inhibited in animals treated with atrazine (200mg/kg) for four days. In addition, we have shown that the inhibition of the LH surge correlates with an absence of cFOS in GnRH neurons. cFOS is the product of an immediate early gene normally expressed in GnRH neurons during the preovulatory surge. The purpose of this study was to determine if the effects of atrazine on GnRH neurons and on the LH surge could be reversed within four days of cessation of atrazine treatment. Ovariectomized, Wistar rats were administered atrazine (200mg/kg/day) or vehicle by gavage for four days. On the third and fourth day, estrogen and progesterone were given in order to induce a LH surge. Half of the animals were transcardially perfused and processed using immunocytochemistry to determine the percentage of GnRH neurons expressing cFOS. Blood samples were taken from the remaining animals during the afternoon/evening. Plasma was assayed for LH using RIA and the amplitude of the LH surge was determined. Atrazine treatment was discontinued in an additional group of animals and the LH surge and the effect on GnRH activation was evaluated four days after the final dose of atrazine to determine if the inhibitory effects of ATRA on LH and GnRH neuronal activation persisted. Results from these studies show Wistar rats treated for 4 days with ATRA had a significant reduction in the magnitude of the LH surge which corresponded to a drop in GnRH neuronal activation. Four days after cessation of ATRA treatment there was no longer a difference between ATRA and vehicle treated animals. These data indicate that ATRA may affect neuroendocrine function in the female Wistar rat by actions at the level of the GnRH neuron; however, these effects are reversed within four days.

Supported by Syngenta Crop Protection Inc.

280 ATRAZINE INHIBITS THE PULSATILE LH RELEASE WITHOUT ALTERING PITUITARY SENSITIVITY TO A GNRH RECEPTOR ANTAGONIST IN FEMALE WISTAR RATS.

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Previous work has shown that exposure of female Sprague-Dawley rats to high doses of atrazine causes a reduction in the pre-ovulatory luteinizing hormone(LH) surge and a disruption of the estrous cycle, resulting in prolonged periods of exposure to endogenous estrogen and the earlier development of mammary tumors. The purpose of this experiment was to determine if the effects of atrazine on the LH surge were due to changes in the pituitary or GnRH function. It is known that the pituitary release of LH can be induced by administering the GnRH receptor agonist, D-ala-6-GnRH (D-Ala-6) in the presence of GnRH antiserum (AS) that removes the endogenous GnRH signal to the pituitary. Wistar rats were ovariectomized and implanted with an indwelling intra-atrial cannula and were administered AS (100ul). Four days later, blood samples (100ul) were taken every 10 min for 100 min. A dose-response to D-ala-6 (10pg, 100pg, 1ng or 10ng) was determined by giving multiple doses of the agonist after the third blood sample by measuring LH levels in blood by RIA. As seen previous in AS treated animals LH levels were below detection in the initial samples due to AS blockage of the effect of endogenous GnRH but rose dramatically after D-ala-6 administration in all groups but the lowest dose group. To evaluate the effect of ATRA on pituitary sensitivity to D-ala-6, ovariectomized Wistar rats were administered ATRA (0, 50, 100, 200 mg/kg) for four days. On the final day of treatment, blood samples were taken every 10 min. for 2 hours. Following the third sample animals were given 100 pg or 1ng of D-ala-6. There were no effects of ATRA treatment on the LH response to either doses of the GnRH receptor agonists. These results suggest that ATRA does not disrupt neuroendocrine function by changing in vivo pituitary sensitivity to GnRH receptor activation.

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281 DIFFERENCES IN METABOLISM AND AROMATASE ACTIVITY IN A FISH AND A RAT SPECIES TREATED WITH ATRAZINE.

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Atrazine, one of the most commonly used herbicides in the United States, is applied to multiple crops such as corn, sorghum, sugarcane, and cotton. The herbicide enters the aquatic environment from run-off after agricultural application, especially in the spring. Atrazine has been shown to disrupt normal reproductive processes in rats, fish and frogs. In vitro evidence suggests that atrazine may interfere with aromatase, the rate-limiting enzyme complex catalyzing the conversion of androgens (androstenedione and testosterone) to estrogens (estrone and estradiol) during steroidogenesis in a wide range of animals. The goal of our research is to determine if effects from exposure to atrazine in one species can predict effects of atrazine exposure in another species. To accomplish this goal, we examined reproductive endpoints and aromatase activity in the brains and gonads of a rat (a model of human health) and a fish (an ecological model) species. Results indicate that the two species show differences in their sensitivity to aromatase modulation by atrazine, with no differences noted in rat aromatase activity or aromatase mRNA in the brain, gonads or adipose tissue following exposure to atrazine, while in fish, brain aromatase activity was significantly elevated. Our results in fish also suggest that atrazine exposure can affect aromatase activity in tissues of the same species differently, because, while aromatase activity in brain was elevated in fish treated with atrazine, no effect was observed in gonads. In addition, differences in the metabolism of atrazine between the two species were elucidated. Through this research, we have identified a number of differences between species that suggest there are uncertainties that need to be considered for atrazine risk assessments across diverse taxa. Research is continuing into the specific mechanisms by which atrazine or its metabolites might impact local aromatase activity and reproductive endpoints in the two species.

282 CHARACTERIZATION OF CYPS IN THE METABOLISM OF ALL TRANS RETINOIC ACID BY LIVER MICROSOMES FROM MICE TREATED WITH CONAZOLES.

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Conazoles are fungicides used in crop protection and as pharmaceuticals. Triadimefon and propiconazole are hepatotumorigenic in mice, while myclobutanil is not. Previous toxicogenomic studies suggest that alteration of the retinoic acid metabolism pathway may involve in conazole-induced hepatotumorigenesis. This study was to examine the atRA metabolism by liver microsomes from conazole-treated mice, and characterize the associated hepatic cytochrome P450 (Cyp) enzyme(s) involved in atRA metabolism. In vitro metabolism of atRA was assessed in liver microsomes from male CD-1 mice following four daily intraperitoneal (IP) injection of propiconazole (210 mg/kg/d), triadimefon (257 mg/kg/d) or myclobutanil (270 mg/kg/d), and total atRA metabolism activity (sum of 4-OH- and 4-oxo-atRA) was quantified with HPLC. Formation of 4-OH-, 4-oxo-atRA, and total

atRA metabolism were significantly increased by all three conazoles. Propiconazole-induced microsomes possessed greater metabolizing activities based on total atRA metabolism and 4-OH-atRA formation compared to myclobutanil-induced microsomes. Cyp26a1, Cyp2b10(20), Cyp3a11, Cyp2c65 and Cyp1a2 genes were significantly over-expressed in the livers from propiconazole- or triadimefon-treated mice, while only Cyp2b10(20) and Cyp3a13 genes were over-expressed in myclobutanil-treated mice. Chemical and immuno-inhibition studies suggested that Cyp26a1, Cyp3a and Cyp2b were involved in atRA metabolism. The over-expression of Cyp26a1, Cyp2b10(20) and Cyp3a11 enzymes are consistent with increased atRA metabolism induced by conazoles in mouse liver. The implications of these changes with respect to reduced atRA levels in hepatic tissues suggest that it might be a necessary event in conazole-induced hepatotumorigenesis. (This abstract does not reflect U.S. EPA Policy).

283 GENDER- AND SPECIES-MEDIATED DIFFERENCES IN THE *IN VITRO* METABOLISM OF TRIADIMEFON BY RODENT HEPATIC MICROSOMES.

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Understanding how metabolism kinetics differ between genders and species is important in developing informative pharmacokinetic models and accurately assessing risk. Metabolism of the conazole fungicide Triadimefon (TDN) was studied in hepatic microsomes of SD rats and CD-1 mice. Michaelis-Menten saturation kinetic parameters were calculated using both substrate depletion and product formation reaction velocities. For values calculated from substrate depletion data, male rat microsomes were found to metabolize TDN most rapidly, with a V_{max} of 1.02×10^4 pmol/min/mg protein, whereas the V_{max} for female rats was the slowest at 1.31×10^3 pmol/min/mg protein. Male and female mice fell intermediate to these values, at 4.31×10^3 pmol/min/mg protein and 4.92×10^3 pmol/min/mg protein, respectively. K_m values calculated for male rat (30 μM), female rat (30 μM), male mouse (50 μM) and female mouse (56 μM) suggest that relevant enzyme affinities were more similar within species than between species. Differences in stereoselectivity of TDN metabolism were also addressed by measuring changes in the enantiomeric ratio with respect to time. These data indicate a need to further investigate differences in metabolic kinetics and mechanisms between species and genders of model organisms in order to develop more accurate pharmacokinetic models for risk assessment.

284 RISK TO HUMANS FROM DIETARY EXPOSURE TO THE NEONICOTINOID INSECTICIDE IMIDACLOPRID.

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Imidacloprid represents the new generation of neurotoxic insecticides, which have more selective toxicity for insects relative to mammals. It is structurally related to nicotine and acts as an agonist of the nicotinic acetylcholine receptor (nAChR). It is registered to control insect pests on agricultural and nursery crops, structural pests and parasites on companion animals. Imidacloprid ranked as one of the top selling pesticides in the world in 2006.
We evaluated the risk for health effects in humans from exposure to residues of imidacloprid in food and drinking water. The toxicity was assessed in animal studies. Two acute oral NOELs (No-Observed-Effect Level) were used to address the acute dietary exposure. The acute NOEL of 9 mg/kg/day for decreases in motor activity in adult rats was used for the general population. The acute NOEL of 5.5 mg/kg/day for developmental neurotoxicity in rats was pertinent to women of childbearing age to protect against fetal exposure. The chronic NOEL of 5.7 mg/kg/day was based on an increase in incidence and severity of mineralized particles in the thyroid gland in rats. Dietary exposures were estimated for 16 population subgroups under acute and chronic scenarios using the deterministic point estimate model. The exposure estimates were based primarily on the maximum allowed residue level (tolerance) as surrogate for residue concentration. The risk was evaluated by comparing the margins of exposure (MOE=NOEL/exposure) to a benchmark MOE of 100. The acute MOEs ranged from 115 to 614 at the high-end (95th–99th) dietary percentiles. The chronic MOEs for all population subgroups were greater than 770. In conclusion, the acute and chronic dietary MOEs for imidacloprid exceeded the general health protective MOE of 100. However, these MOEs reflected only the risk from the dietary exposure. Additional exposures from ambient air, occupational activities and residential uses will lead to reductions in the aggregate MOEs.

285 DETERMINANTS OF HUMAN EXPOSURE TO PET PEST PRODUCTS: FIPRONIL.

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Fipronil (5-Amino-1-(2, 6-dichloro-4-(trifluoromethyl) phenyl)-4-(1,R,S)-(trifluoromethyl)sulfinyl)-1H-pyrazole-3-carbonitrile; CAS Number 120068-37-3) is an important insecticide registered in at least 47 products for pest management. Pet owners often protect dogs and cats from fleas with topical applications of liquid formulations containing 9.8% a.i. (rates up to 5 mg fipronil/kg). The extent of human exposures resulting from these applications has not been reported. Pilot studies have used spot urine specimens from persons familiar with our biomonitoring research who have inquired about the extent of their fipronil exposure. Acid hydrolysates of urine contain microgram amounts of the corresponding sulfone metabolite (<30 – 400 μg/L). Potential biomarkers appeared within 1 day of use of fipronil and urine elimination of progressively smaller amounts sulfone continues for several days to 2 weeks. Primary contact with treated pets and secondary contact with indoor residues are likely based upon previous unpublished studies with chlorpyrifos pet products. Direct skin contact may be an important determinant of fipronil exposure. The potential for inhalation exposure is being surveyed using an electrostatic air filtration device (Oreck, New Orleans, LA). The most common measure of potential pet product exposure has been cotton glove retention of residues. Latex gloves may be a superior dosimeter. Analysis of latex gloves showed time-dependent levels of fipronil and other fipronil products (ranging from 1,234 μg/pair to < 66 μg/pair). Other contaminated surface residues surveyed as potential source(s) of pet owner exposure include textiles used as bedding, floor coverings including carpet and linoleum, and pet bedding. Future studies will clarify any relationship between surface residues, particularly glove levels, and pet owner fipronil exposure.

286 CHLORPYRIFOS ALTERS METHYLATION STATUS OF PROMOTER-CPG ISLANDS IN PLACENTAL CHORIOCARCINOMA CELLS.

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The mechanism by which chlorpyrifos (CPF) exerts its toxicity in fetal and perinatal animals has yet to be fully identified. Since the placenta is responsible for transport of nutrients and is a major supplier of hormones to the fetus, exposure to xenobiotics which interfere with the function and/or viability of placental cells could ostensibly alter the development of the fetus. Our laboratory has recently demonstrated that CPF induces dose-dependent reduction in cellular viability and alters the gene expression of several genes which regulate apoptosis in placental cells. It should be noted that gene expression is often influenced by methylation status, whereas DNA hypomethylation induces gene expression and hypermethylation induces gene silencing. Given that CPF exposure can alter gene expression, we studied whether the effects may be related to changes in the methylation status of promoters containing CpG islands. Of the approximately eighty (80) promoters surveyed, only PgA, SURVIVIN, VHL and IRF7 genes were found to be methylated in control (drug vehicle) cells; however, CPF treatment enhanced the methylation of these aforementioned genes and resulted in the hypermethylation of an additional thirteen (13) genes. These included ATF2, BAGE, HOXA2, IL-4, MASPIN, MYC-L2, NES-1, PAI-1, PgA, RPA2, TASTIN and TFF1. Taken collectively, these results suggest that CPF can alter gene expression by modifying the methylation status of CpG rich promoters. Furthermore, these hypermethylated genes may serve as potential biomarkers of CPF-related toxicity.

287 BASIC COMPONENTS OF WOOD SMOKE PARTICULATE MATTER CAUSE OXIDATIVE STRESS AND CASPASE-DEPENDENT APOPTOSIS IN RAW 264.7 CELLS.

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We previously reported that treatment with 25ug/ml, 50ug/ml and 100 ug/ml of neutral, acidic, and unfractionated (total) wood smoke extracts cause cytotoxicity after 24 hours in RAW 264.7 (RAW) cells in a dose-dependant manner. Basic extracts (acidic components) were not cytotoxic at the same doses. Flow cytometry analysis with Annexin V and propidium iodide indicated cellular death via apoptosis. Assays for caspase 3 were positive at 12 hours for all doses of unfractionated extract, suggesting the mode of death is caspase-dependent apoptosis. Western blot for procaspase 3, and cleaved caspase 3 products and cleavage of a caspase 3 target, the glutamate cysteine ligase catalytic subunit (GCLc), confirm activation of caspase 3 at the 50ug/ml and 100ug/ml doses. Glutathione levels were upregulated in

RAW cells treated with 10, 25 or 50 ug/ml unfractionated extract. Treatment at the 100 ug/ml dose resulted in glutathione depletion. Glutamate Cysteine Ligase (GCL), the rate-limiting enzyme in glutathione synthesis, was upregulated in a dose-dependent manner for all doses of unfractionated extract. These data suggest the basic components of campfire wood smoke appear to cause oxidative stress and induce apoptosis through a caspase-dependent pathway in RAW cells.

288 UPREGULATION OF POLYAMINE BIOSYNTHESIS IN RAT LUNG FOLLOWING BLEOMYCIN ADMINISTRATION.

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Bleomycin (BL) is an antineoplastic drug used for treatment of squamous cell carcinomas and lymphomas, but causes pulmonary fibrosis (PF) as a side effect. The biogenic polycationic amines, polyamines (PA) involved in cellular growth and differentiation, inflammation, and carcinogenesis, are terminal carboxamide substituants of BL. Studies to modulate BL toxicity using difluromethylornithine to inhibit orinithine decarboxylase (ODC), the rate-liming enzyme of PA biosynthesis, were unsuccessful. In this study, we examined PA and hydroxyproline (HP), a biomarker of collagen synthesis, to characterize the time-course of changes after BL administration, and whether PA can serve as biomarkers of BL-induced PF. We administered BL to Sprague-Dawley rats (1.5 unit/rat) by intratracheal instillation daily for 1, 7, 14 or 28 days. We found that PA metabolic pathway constituents including ODC and S-adenosylmethionine decarboxylase (AdoMetDC) activities, and putrescine (PUT) spermidine (SPDN) and spermine (SPN) contents, were significantly upregulated at all time points after BL administration compared to saline control reaching a peak on Day 7. In contrast, HP content was significantly elevated in rat lung only on Day 28 (64%, $P<0.05$). Lung weight (both absolute and relative to body weight) and protein content were statistically greater from Day 7. The results indicate that BL administration causes rapid and significant upregulation of PA metabolism, but slower accumulation of HP suggesting that early downregulation of PA may slow HP formation and thus reduce BL toxicity.

289 INTERMEDIATE FILAMENT PROTEINS AS TARGETS FOR REACTIVE CARBONYL COMPOUNDS.

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Intermediate filaments (IF) are dynamic components of the cytoskeleton and also act as substrates for several signal transduction effectors involved in cell growth and death. To date, few studies have addressed IF proteins as molecular targets for acrolein, a highly reactive aldehyde associated with numerous degenerative diseases and drug-induced toxicities. In this study, the effects of increasing concentrations of acrolein (30, 100, 300, 1000 μM) on cell viability and IF protein carbonylation were investigated using human alveolar basal epithelial cells (A549). In addition, mass spectrometry was carried out to ascertain the identities of carbonylated IF proteins. Acrolein reduced cell viability in a dose-dependent manner during a two hour exposure but only 1000 μM was significantly different from controls ($p<0.001$). However, acrolein caused a concentration-dependent increase in IF protein carbonylation from 30 to 1000 μM as detected by immunoblotting. Mass spectrometry analysis revealed that five IF proteins were specifically carbonylated by acrolein, namely vimentin (MW 53), cytokeratin 8 (MW 53), cytokeratin 7 (MW 51), cytokeratin 18 (MW 47) and cytokeratin 19 (MW 44). Densitometry performed on immunoblotting images and Coomassie blue-dyed gels revealed that if the density of each carbonylated IF protein band was determined as a fraction of its total amount of protein expressed, vimentin was the most carbonylated IF protein followed by cytokeratin 8, cytokeratin 18, cytokeratin 7 and cytokeratin 19. Similar trends were observed following exposure of cells to crotonaldehyde and methyl vinyl ketone, which are close structural analogues of acrolein, although these compounds produced less reduction in cell viability and lower yields of carbonyl adducts compared to acrolein. These findings suggest that the extent of IF protein carbonylation induced by a given concentration of carbonyl compound was closely correlated to its toxicity and that its specificity opened up several areas for future exploration as vimentin, cytokeratin 8 and 18 had been shown to participate in cell death regulation.

290 CARBONYL SCAVENGERS SUPPRESS THE TOXICITY OF POLYETHYLENE PYROLYSIS PRODUCTS.

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The combustion of polyethylene (PE) and other plastics generates the noxious 3-carbon electrophile acrolein, a key contributor to smoke inhalation injury (SII). Unfortunately, existing pharmacological treatments for SII are not rationally directed against the pathogenetic role of acrolein or other toxic smoke constituents. The purpose of this study was to evaluate carbonyl scavengers with known abilities to suppress acrolein toxicity for efficacy against the toxicity of PE pyrolysis extracts. The latter were prepared by bubbling smoke produced during combustion of PE (5 g) through a buffered saline trap. Acrolein concentrations in the extracts were determined via HPLC after derivatization with 2,4-dinitrophenylhydrazine. PE pyrolysis extracts were added to A549 cell monolayers to final acrolein concentrations of 25 μM, while carbonyl scavengers were tested at 10, 25, 50, 100 or 200 μM concentrations. The carbonyl scavengers assessed were bisulfite, D-penicillamine, α-lipoic acid, hydralazine and its analogue 1-hydrazinoisoquinoline. Cell viability was assessed by estimating ATP levels (Promega CellTiter Glo Luminescent assay). Exposure to PE pyrolysis extract alone reduced ATP levels to 36 ± 11% of controls after 4 h (mean ± SE, N = 4). Bisulfite was the most efficient inhibitor of toxicity, with 50, 100 and 200 μM concentrations maintaining ATP levels at 52 ± 16, 97 ± 12 and 103 ± 5 % relative to controls. The other carbonyl scavengers also suppressed toxicity at the highest concentrations tested. To determine whether bisulfite prevented damage to intracellular targets, carbonyl adducts were measured in intermediate filament (IF) proteins isolated from A549 cells following 1 h exposure to smoke extracts. Four IF proteins, vimentin and cytokeratins-7, -8 and -18, were conspicuous targets for carbonylation. Protective concentrations of bisulfite attenuated carbonylation of the cytokeratins and to a lesser extent vimentin. These findings suggest carbonyl-trapping drugs may deserve further investigation as possible SII treatments.

291 A GENETIC BASIS FOR THE HEIGHTENED SENSITIVITY OF THE NEONATAL MOUSE LUNG TO OZONE.

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Ozone is a powerful oxidant and respiratory irritant that leads to airway inflammation and pulmonary dysfunction. Although studies have not established a greater susceptibility to ozone in children, rodent studies have shown that neonates are more sensitive than adults. Using a murine model, we tested the hypothesis that there is a genetic basis for the differential response of neonatal and adult lungs to inhaled pollutants. Our initial studies not only showed a heightened sensitivity to ozone in neonates in comparison to adults, but also revealed clear and quantitative interstrain differences in response to ozone in neonates. In our current study, we executed a classic intercross F2 study to identify quantitative trait loci (QTLs) associated with the variable response of neonatal mice to ozone. Using resistant (A/J) and sensitive (Balb/cJ) mouse strains, we generated over 100 F2 progeny which were exposed (15 to 16d old) to 0.8ppm Ozone for 5h. Inflammation and pulmonary injury were evaluated in lavage fluid 24-hours post-exposure. Kidney DNA was genotyped and linkage analysis was performed. Linkage analysis revealed a significant QTL on chromosome 7 and two suggestive QTLs on chromosome 14. Furthermore, it was observed that F1 progeny inherited resistance (A/J-like) to lung inflammation but susceptibility (Balb/cJ-like) to lung injury. These results strongly suggest that genetic determinants play an important role in the enhanced sensitivity of the young mammalian lung to ambient air pollutants. Moreover, detailed examination of chromosomes 7 and 14 coupled with gene chip expression data may reveal candidate genes that play a critical role in the differential response of the juvenile lung to ozone. A second F2 study has also been completed using different resistant (129x1/SvJ) and sensitive (SJL/J) strains and a haplotype mapping experiment involving 30 inbred mouse strains is underway to further elucidate the specific genetic factors contributing to ozone susceptibility and lung toxicity.

292 THE INORGANIC FRACTION OF PM_{10} AND $PM_{2.5}$ INDUCES OXIDATIVE STRESS IN HUMAN LUNG CELLS.

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Exposure to airborne particulate matter (PM) has been associated with cardiopulmonary toxicity involving oxidative stress from reactive oxygen species (ROS) generated by inorganic (metals) or organic compounds. In this study ambient air particles, PM_{10} and $PM_{2.5}$ collected in Mexico City, were subjected to heat treatments to eliminate organic compounds and separate the inorganic component. PM were analyzed for their metal content by induced coupled plasma, elemental and organic carbon (EC/OC) by TOT-NIOSH and oxidative activity by the DTT assay. To assess the contribution of PM inorganic and organic compounds in oxidative stress induction and DNA damage human alveolar epithelial A549 cells were exposed to 20, 40 and 80μg/cm^2 of treated (inorganic fractions) and untreated PM_{10} and $PM_{2.5}$. Intracellular ROS generation, glutathione (GSH) depletion and the formation of 8-oxoguanine DNA adduct were measured. PM_{10} contained higher

amounts of metals (Cr, Fe, Mn, Ni, Ti, Zn and V) than $PM_{2.5}$, similar amounts of OC were observed in PM_{10} and $PM_{2.5}$, and EC was higher in PM_{10}. $PM_{2.5}$ showed higher redox activity than PM_{10}. The inorganic fractions from both PM samples contained higher amounts of metals, elemental carbon and showed no redox activity. Intracellular ROS generation was induced by all PM types in a concentration dependent manner, the inorganic fractions being more powerful. GSH depletion was significantly higher with the inorganic fractions followed by PM_{10} and by $PM_{2.5}$. No DNA damage was observed with any of the PM treatments. The inorganic fractions of PM showed more metals and EC, no oxidative potential and a higher effect in the induction in ROS generation and GSH depletion, suggesting a determinant role of inorganic components in the induction of oxidative stress and PM-related toxicity.

293 AIRBORNE PARTICULATE MATTER DEPLETES GLUTATHIONE AND INDUCES CELL DEATH ON HUMAN ALVEOLAR CELLS.

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Airborne particulate matter (PM) is a well known cardiopulmonary toxicant. One of its most important toxic effects is the induction of oxidative stress in different cell types and tissues. In this study we investigated how human lung epithelial carcinoma cells (A549) react to particulate matter toxic effects regarding reactive oxygen species (ROS) production, glutathione-mediated antioxidant response and the induction of cell death or proliferation. Cells were exposed to different concentrations (10 $\mu g/cm^2$ – 100 $\mu g/cm^2$ for 6, 12 and 24 h) of ambient air particulate matter (PM_{10} and $PM_{2.5}$) collected in the north of Mexico City. Intracellular ROS production was measured by a fluorescence assay using dichlorofluorescein diacetate oxidation as a molecular probe. Reduced glutathione (GSH) was determined by a colorimetric method and to assess cell damage inflicted by PM to A549 cells, the ATP and ADP fluctuation was evaluated at 48 h to determine whether cells die via apoptosis, necrosis or they undergo proliferation. Results showed a concentration and time-dependent ROS production and GSH depletion after PM exposure regardless of PM size. Cell death via necrosis was observed after exposure to PM, PM_{10} induced secondary necrosis in a dose-dependent manner (20, 40 and 80 $\mu g/cm^2$) whilst $PM_{2.5}$ induced necrotic cell death drastically only with 80 $\mu g/cm^2$ exposure. Our results suggest that a disruption in intracellular redox state from PM exposure promotes cell death by necrosis, and could also increase the susceptibility of cells to subsequent damage.

294 GENE PROFILING OF LIBBY AMPHIBOLE ASBESTOS COMPARED TO CROCIDOLITE ASBESTOS IN HUMAN MESOTHELIAL CELLS.

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Vermiculite ore contaminated with amphibole asbestos has been mined in the Libby, Montana region since the 1920s, and its processing has led to exposure in nearly 200 sites across the U.S. Recent evidence suggesting extremely low levels of Libby asbestos may cause mesothelioma, along with the fact that there is a prolonged latency phase prior to disease onset, lends urgency to understanding early molecular events. Studies presented here use microarray analysis to compare gene expression profiles of Libby asbestos with crocidolite asbestos, a well characterized asbestos type both mechanistically and pathologically which served as a positive control. Telomerase-immortalized human mesothelial cells (LP9) were treated with equal surface area concentrations of Libby or crocidolite asbestos (NIEHS reference sample) for 8 or 24 h, and differences in gene expression were documented using Affymetrix GeneChip HG-U133A 2.0 arrays targeting 18,400 human transcripts. Data were analyzed using GeneSifter software. At 8 h, Libby asbestos elicited increases of >3-fold in 1 gene (superoxide dismutase 2; SOD2) compared to 29 significant gene changes caused by crocidolite asbestos treatment. At 24 h, crocidolite asbestos caused nearly twice as many gene changes as Libby asbestos (205 versus 111, respectively). Of the 111 changes observed following Libby asbestos exposure, approximately 67% involved transcriptional upregulation of genes related to signal transduction, reactive oxygen species (ROS) metabolic processes, and immune response, among others. Since upregulation of SOD2 was observed at both 8 and 24 h, we further examined the involvement of ROS in the mechanism of toxicity of Libby asbestos. These studies not only demonstrate that Libby asbestos produces significantly less gene changes than crocidolite asbestos, but also identify potential targets for therapeutic intervention of asbestos-induced mesothelioma. Supported by NIEHS T32ES0071.

295 MITOCHONDRIAL OXIDATIVE AND ENZYMATIC DAMAGE IN ACUTE CARBON MONOXIDE POISONING: LIPID PEROXIDATION AND COMPLEX IV ACTIVITY IN LYMPHOCYTES AND ITS MODIFICATION AFTER OXYGEN THERAPY.

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Background: Mitochondrial damage could be relevant in the development of symptoms and response to oxygen therapy in acute carbon monoxide (CO) poisoning (ACOP). Objective: To establish in ACOP patients 1) lipid peroxidation; 2) mitochondrial complex IV (mCIV) activity; 3) improvement depending on therapy (normobaric oxygen -NBO- vs hyperbaric oxygen -HBO-) and 4) correlation with intoxication severity. Patients: 30 subjects with ACOP were distributed as: a) severe (COHb>20%, n:15), randomly treated with one or two HBO sessions (SHBO1 and SHBO2), and b) moderate (COHb: 10-20%, n:15), randomly treated with NBO (MNBO) or one session of HBO (MHBO1). Controls: 26 healthy volunteers. Method: 1) carboxihemoglobin (COHb) levels; 2) lipid peroxidation (malondialdehyde and 4-hydroxyalkenate measurement through spectrophotometry); 3) mCIV activity (spectrophotometry) in lymphocytes (Ficoll technique), before and immediately after the treatment, as well as at 24 h, 1 month and 3 months later. Analysis of results: Comparison of parameters: 1) between MNBO-MHBO1, SHBO1-SHBO2 and MHBO1-SHBO1 groups; 2) evolution for each treatment group; 3) among groups at different time-points. Results: We did not find differences in lipid peroxidation between groups, neither along the time. mCIV activities were decreased in severe and moderate ACOP compared to controls ($p = 0.005$ and 0.033, respectively), but were not different to immediately after-treatment values. We did not find differences in after-treatment mCIV activities between MNBO and MHBO1, nor between SHBO1 and SHBO2. Recovery of mCIV activity along time was significative in SHBO1 and SHBO2 ($p=0.015$ and 0.048, respectively), but not in MNBO or MHBO1. Conclusions: In severe and moderate ACOP there is a decrease in mCIV activity but lipid peroxidation is not increased. One single session of HBO is enough effective in restoring mCIV activity in severe ACOP (FIS PI041239).

296 BIOMARKERS OF SULFUR MUSTARD EXPOSURE IN HUMAN CELL LINES.

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Sulfur mustard is a likely agent for chemical attack, based on its ease of synthesis and dispersal. Furthermore, it is odorless and colorless, and causes no acute distress. Its use in the Iraq-Iran war led to delayed and persistent lung pathology in patients exposed by inhalation. In order to define the pathophysiological mechanisms involved in the response to this potential chemical treat agent, we treated three types of human cell lines with a wide range of doses of purified sulfur mustard. The cells used were a human lung epithelial line with characteristics of Clara cells (H441), a human monocyte cell line (THP-1), and a human lung fibroblast cell line (LL86). Cytotoxicity was measured using both release of lactate dehydrogenase and alterations in tetrazolium cleavage (WST-1). Cytokine release was determined using a multiplex assay for 30 human cytokines, chemokines, and growth factors. Metalloproteinases were measured using multiplex assays as well as zymography. Heme oxygenase-1 was measured as an indicator of oxidative stress. The results indicated toxicity at high doses, and altered levels of some cytokines, metalloproteineases, and HO-1 at sub-toxic doses, that varied with the cell type.

297 GAS-PARTICLE PARTITIONING IN URBAN AIR MIXTURES AND ITS EFFECT ON HUMAN EPITHELIAL LUNG CELLS.

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Both gasoline and diesel motor vehicles are known significant sources of ambient air toxics and PM. Vehicle emissions play a critical role in generating ozone and other photochemical oxidants. More importantly, previous studies performed with diesel emissions (DE) have shown that fresh DE (gases and particles) can undergo significant atmospheric reaction in a few hours, that aging adds mass to the particle phase, and that the increase in mass coincides with an increase in polarity. In addition, our prior *in vitro* cell exposure work suggests that gas-phase polar organic compounds tend to induce greater inflammatory responses than non-polar compounds.
The goal of this study is to determine cellular responses to photochemically-aged urban air mixtures both in the presence and absence of PM. Both DE and ammonium sulfate seed particles were aged in the presence of 55+ VOCs, representing a

typical urban atmosphere. Examining air mixtures with PM present allowed semi-volatiles to partition from the gas phase to the particle phase. This change in composition was measured for each phase, as was the resulting change in toxicity. The relative toxicity of both the gas and particle phases of each air mixture were measured by exposing human epithelial lung cells to gas and particle phase components separately. IL-8, a marker of inflammation, was measured using standard ELISA and RT-PCR assays. Cytotoxicity was analyzed by monitoring LDH release.
Our findings indicate that the presence of PM in reaction mixtures significantly reduced the concentration of oxygenated species in the gas phase of the aged mixtures, which resulted in a decrease in the cellular response to the aged gas-phase exposure. Conversely, the toxicity of the particle phase increased from fresh to aged PM. These results support the assertion that the more-polar toxic compounds are partitioning to the particle phase as they age in the atmosphere, and that these compounds contribute to the observed toxic response.

298 INHALATION OF COMBUSTION-DERIVED NANOPARTICLES STIMULATES EXPRESSION OF NRF2 MEDIATED OXIDATIVE STRESS RESPONSE GENES IN MURINE LUNG.

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Rationale: Incomplete combustion of the high-volume industrial petrochemical 1, 3-butadiene (BD) yields a broad range of potential respiratory system toxicants, including polynuclear aromatic hydrocarbons (PAHs), adsorbed to the surface of the ultrafine BD soot (BDS). We have previously described the physicochemical characteristics of BDS and reported neutrophilic infiltration and soot (free and phagocytized) in the airways of mice exposed to BDS. We now describe gene expression changes in the lungs of adult Balb/c mice initiated by BDS inhalation. Methods: Male Balb/c mice were exposed by inhalation to BDS (5 mg/m3; 4 hrs/day; 4 days) and sacrificed one hour following final BDS exposure. One lung was collected for total RNA isolation. We examined, via Affymetrix Mouse Genome 430 2.0 Arrays, gene expression profiles in these lungs. Data sets were filtered by transcript p-value (≤ 0.05), false discovery rate (≤ 0.05), and fold change (≥ 1.5). Results: A total of 261 differentially regulated transcripts were identified (71 down-regulated and 190 up-regulated). Major pathways associated with the differentially regulated genes included aryl hydrocarbon receptor and xenobiotic metabolism signaling (Cyp1a1, Cyp1b1, Aldh3a1, Ahrr); oxidative stress/Nrf2 mediated oxidative stress responses (Fos, Hmox1, Nqo1, Nrf2); IL-10 signaling (Bcl3, Cd14, Il4r). Conclusion: Gene expression differences seen as a result of exposure to BDS are consistent with previously reported histopathological changes. Gene expression data and histopathology reflect the previously described character (fine particles; PAH's) of BDS. In addition to aryl hydrocarbon receptor activation, inflammation, and xenobiotic metabolism, BDS exposure initiates Nrf2 mediated oxidative stress responses.

299 IMPAIRMENT OF MACROPHAGE FUNCTION UNDER MODERATE CONCENTRATIONS OF OXYGEN.

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Mechanical ventilation with supraphysiological concentrations of oxygen (hyperoxia) is routinely used to treat critical ill patients. However, a significant number of the patients on ventilator develop ventilator-associated pneumonia. We have shown that administration of > 90% oxygen can cause acute lung injury, accompanied by the impaired phagoctotic ability of alveolar macrophages. In this study we examined the effect of moderate concentrations of oxygen on macrophage's functions that are essential in bacterial clearance. Exposure to 80, or 60, or 40% oxygen for 24 hours resulted in significant suppression of macrophages' ability to migrate and to phagocytose, although no cell death was observed in these macrophages. In addition, exposure to these concentrations of oxygen reduced LPS-induced NO release from the cultured macrophages, suggesting that even the moderate concentrations of oxygen causes macrophage dysfunction in bacterial clearance. Corresponding to the macrophage dysfunction, actin carbonylation and disruption of actin cytoskeleton were increased in hyperoxic macrophages, which is correlated with a decrease in glutathione availability in these cells. Administration of procysteine which can increase the levels of glutathione precursor, normalized the levels of reduced glutathione and restored macrophages function. These results suggest that exposure to even moderate concentrations of hyperoxia can increase oxidative stress, underlying the mechanisms for the increased actin oxidation and the impairment of macrophages to effectively migrate and phagocytose. The decreased NO-release in response to LPS in hyperoxic macrophages may further hinder the effectiveness of macrophages to clear bacteria.

300 DOES SANDBLASTED METAL ATTENUATE OR ENHANCE THE TOXICITY OF FRESHLY FRACTURED SILICA?

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Trace metals are reported to play important roles in catalyzing the formation of highly reactive oxygen species (ROS), which are implicated in enhanced disease development. Whether trace metals from a concrete cutting saw increase the toxicity of silica is unclear. In this study, pure metal plates of aluminum (Al), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), nickel (Ni), tin (Sn), and tungsten (W), all used in making saw blades, as well as a saw blade itself were blasted with silica using an automated sandblasting apparatus. The blasting produced an aerosol of silica dust containing measurable amounts of blasted metals. Aerosolized samples were analyzed and evaluated for in vivo acute toxicity, DNA damage, activation of AP-1 and NF-κB, and potential to generate ROS. The results show that inflammatory response and pulmonary toxicity 3 days post-exposure associated with silica blasted on Sn, Ni, Saw, Cu and W being greater than silica alone. In contrast assimilation of metals, such as Cr, and Al, significantly decreased the toxicity of silica. The potential to generate ROS using a non-cellular system did not correlate with the degree of toxicity. For example, saw blade blasted with silica caused a high level of toxicity but a relatively low level of ROS generation. This is likely because metals, such as Ni, Co, and Cr, can produce greater levels of ROS in a biological milieu with reductants than in a noncellular system. Sn, saw blade, Cu and Co blasted with silica caused significantly greater levels of DNA damage compared to silica alone. The potential for DNA damage did not follow that for ROS production. Saw blade blasted with silica also caused significantly greater levels of activation of two important transcription factors, AP-1 and NF-κB, involved in disease development. These data suggest a potential increased risk associated with silica dust generated using concrete cutting saw.

301 FROM XENOBIOTICS TO ENDOBIOTICS: EFFICIENT HYDROLYSIS OF THE ENDOCANNABINOID 2-ARACHIDONYLGLYCEROL BY HUMAN CARBOXYLESTERASES 1 AND 2.

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Carboxylesterases (CEs) have important scavenger and catalytic roles in pesticide metabolism and contribute to xenobiotic clearance. The tissue with the highest expression of CEs is the liver, befitting their role in xenobiotic metabolism. However, CEs also have important functions in lipid metabolism and appear to harness its broad substrate specificity to metabolize not only xenobiotics but also endobiotics involved in health and disease. The aim of this study was to determine whether human CE isoforms hCE1 and hCE2 catabolize the endocannabinoids 2-arachidonylglycerol (2AG) and anandamide (AEA), two important lipid signaling mediators. Using HPLC/MS to assay production of arachidonic acid, we show that recombinant hCE1 and hCE2 proteins efficiently hydrolyze 2AG, but not AEA. Hydrolysis of 2AG was time-, protein-, and 2AG concentration-dependent. The steady-state kinetic parameters obtained for hCE1 and hCE2, respectively, are: k_{cat}, 59 and 43 min^{-1}; K_m, 49 and 46 μM; k_{cat}/K_m, 1.2 and 0.9 μM^{-1} min^{-1}. These data indicate that hCE1 and hCE2 both exhibit very similar catalytic efficiencies for 2AG hydrolysis, and the obtained k_{cat}/K_m values are comparable to published values for monoacylglycerol (MAG) lipase and fatty acid amide hydrolase (FAAH), two enzymes known to regulate 2AG levels in vivo. Further, intact human THP1 monocytes, which express hCE1 but not hCE2, were treated with the organophosphate inhibitor paraoxon (10 μM) to examine the contribution of hCE1 to 2AG catabolism in vitro. Lysates from paraoxon-treated cells metabolized fortified 2AG (100 μM) at significantly slower rates compared to vehicle-treated cells (~30% reduction, $p<0.05$). Collectively, these results suggest that in addition to the known pharmacological targets MAG lipase and FAAH, CEs may also be attractive targets for selective inhibitors in order to elevate endocannabinoid levels in the body. [Funded in part by NIH P20RR017661 and ALSAC]

302 METHYLATION OF DIMETHYLTIN IN MICE AND RATS.

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Organotins are widely used as stabilizers of polyvinyl chloride, and as catalysts or biocides. It is well known that dimethyltin (DMT) is less neurotoxic than trimethyltin (TMT). A Korean worker who was exposed to DMT compounds

showed neurological symptoms similar to those of TMT encephalopathy, in association with high levels of both DMT and TMT in the urine and blood. The case suggested the possibility of methylation of DMT in humans. Here, we investigated if TMT is detected in the urine of mice and rats exposed only to DMT dichloride (DMTC). Three Slc:ICR mice and three Slc:wistar rats were placed in individual metabolite cages; and one day later, they were injected intraperitoneally with DMTC (10 mg/kg body weight (wt); 5.4 mgSn/kg body wt; 45.5 µmol/kg body wt) over 4 consecutive days. Twenty-four hour urine samples were collected every evening for 11 consecutive days starting at baseline (before treatment). Speciation analyses of methyltin compounds in urine were performed using a combination of high performance liquid chromatograph-inductively coupled plasma mass spectrometry. High concentrations of DMT and time-dependent increase of TMT were found in both mice and rats during the 4-day treatment, and their concentrations decreased gradually after cessation of treatment. The chemical compound of the detected peak was confirmed to be TMT by liquid chromatography-tandem mass spectrometry. Neither DMT nor TMT was detected in the samples collected at baseline. Our results indicate urinary excretion of TMT in mice and rats exposed to DMTC, confirming the production of TMT in vivo, probably through methylation of DMT.

303 EFFECT OF OSMOTIC STRESS ON THE EXPRESSION OF FLAVIN-CONTAINING MONOOXYGENASE mRNA, PROTEIN AND CATALYTIC ACTIVITY IN RAT KIDNEY.

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Flavin-containing monooxigenases (FMOs) are a polymorphic family of enzymes that catalyze the oxidation of soft nucleophilic heteroatom substances to their respective oxides. Previous studies in euryhaline fishes indicated induction of FMO expression and activity in vivo under hypersaline conditions. In this study we evaluated the effect of hypersaline conditions on FMO expression in rat kidney. Three hours after an intraperitoneal injection with 3.5 M HEPES-buffered saline at a dose of 0.6 cc/ 100g, male Sprague-Dawley rats, FMO activities, as well as expression of mRNA and protein was evaluated in kidneys. A positive significant correlation was found between FMO1 protein expression and plasma osmolarity. FMO activities using thiourea and methimazole oxidase were unaltered by treatment. However, a statistically significant increase in methyl-p-tolyl sulfide oxidase was observed after treatment. A positive significant correlation between plasma osmolarity and FMO1-catalzyed (R) methyl-p-tolyl sulfoxide was also observed. There were no changes in FMO1, FMO3 or FMO5 transcripts or FMO3 and FMO5 protein content in kidneys. These results suggest that expression of FMO1 in kidney may be affected by osmotic stress, but through a non-transcriptional regulatory pathway. Supported by UCTSR&TP.

304 MECHANISM OF METHYLMERCURY BIOTRANSFORMATION IN HUMAN CELL LINES.

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Methylmercury (MeHg), an environmental neurotoxicant, is gradually transformed to inorganic Hg *in vivo*. This biotransformation is an important factor for the evaluation of MeHg toxicity in organisms, particularly in a chronic phase. In present study, we examined the demethylation of MeHg in human hepatocytes (Chang Liver), human glioblastoma (U373MG) and human neuroblastoma (SK-N-SH). Based on the MeHg toxicity to the cell growth, Chang Liver and U373 MG were exposed for 24, 48 and 72 h to 1, 2, 3 or 5 µM MeHg. SK-N-SH, the most sensitive cells, was exposed only to 1 µM MeHg. MeHg uptake occurred in dose-dependent manner and approached a plateau level within each cell line at 24 h after MeHg exposure. Total Hg concentration was the highest in SK-N-SH with 1 µM MeHg exposure. The cellular inorganic Hg concentration increased gradually up to 72 h after MeHg exposure. The correlation between MeHg accumulation and inorganic Hg production demonstrated that the ability of MeHg biotransformation was highest in Chang Liver. Since the demethylation of MeHg in rat liver slice was reported to proceed with the aid of superoxide anion produced in the electron transfer system at the mitochondrial inner membrane, the effects on MeHg demethylation of several reagents that could modify reactive oxygen production were examined in the human cell lines. Methylviologen which stimulates superoxide anion production was found to be an effective enhancer of demethylation with only a minor effect on lipid peroxidation. On the other hand, ferrous ion showed no effect on the demethylation in the presence of methylviologen, although lipid peroxidation levels were increased significantly. Furthermore, mannitol, hydroxyl radical scavenger, had no effect on inorganic Hg production. These results suggested that superoxide anion, not hydroxyl radicals may participate in demethylation of MeHg.

305 PURIFICATION AND CHARACTERIZATION OF FLAVIN-CONTAINING MONOOXYGENASE ISOFORM 3 (FMO3) FROM MALE RAT KIDNEYS.

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Rats are a common animal model for metabolism and toxicity studies. In previous studies, the enzymatic properties of rat FMOs were examined after the purification and characterization of FMO1 from hepatic and renal tissues and FMO3 from hepatic tissues. Additional studies detected FMO3 by western blot in rat renal microsomes at levels corresponding to methionine S-oxidase activity indicating that FMO3 is present and active in the rat kidney. The purpose of this study was to further characterize the enzymatic properties of renal FMO3 by purification from male rat kidneys and to determine the kinetic parameters with several substrates. FMO3 was purified via affinity columns based on methionine S-oxidase activity and reactivity with human FMO3 antibody. Typically, methionine S-oxidase specific activity was increased 100 fold through the purification processes. The resulting protein band had an apparent molecular weight of approximately 60 kDa by SDS-PAGE lining up closely with a cDNA expressed human FMO3 standard. When the protein band was subjected to trypsin digestion and MALDI-TOF mass spectral analysis, peptides corresponding to over 20% sequence coverage of rat FMO3 were detected. The purified kidney FMO3 was used to determine the apparent Km and Vmax with L-methionine, seleno-L-methionine, S-allyl-cysteine and methimazole. The diastereometric ratios of the resulting sulfoxides were determined by HPLC for L-methionine, seleno-L-methionine, and S-allyl-cysteine. The results show that kinetic values were similar in magnitude to values previously reported for rat liver FMO3. Collectively, these results suggest that FMO3 may play an important role in the metabolism of xenobiotics containing sulfur and selenium atoms in the rat kidney. (Supported by NIH DK44295 and NIEHS T32-ES-007015)

306 EFFECT OF MICROBIALLY INDUCED TRANSFORMATIONS OF BROMINATED FLAME RETARDANTS AND TOXICITY IN ZEBRAFISH.

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Tetrabromobisphenol A (TBBPA) is the most prevalent brominated flame retardant, used mainly in the production of epoxy and polycarbonate resins for circuit boards. Concern has escalated in recent years due to the accumulation of these chemicals in the environment due to their lipophilic properties and persistent nature. TBBPA is reductively dehalogenated to bisphenol A (BPA) in anaerobic sediments and O-methylated to the dimethyl ether form in aerobic sediments. The microbial induced transformations create metabolites with radically different chemical structures as compared to TBBPA and the effect of these transformations on the degradation process and toxicity are not well understood. Our hypothesis is that microbial communities from TBBPA contaminated sites have the ability to dehalogenate and/or O-methylate TBBPA. Further, the microbial transformation products can be more or less toxic to eukaryotic organisms. Sediments were collected from NJ/NY sites, spiked with 10uM TBBPA, observed for heterotrophic growth and analyzed by GC-MS for the presence of TBBPA dimethyl ether. O-methylation has been observed thus far in sediments from Kearny Marsh, NJ. To analyze the differences in toxicity, zebrafish (Danio rerio) embryos were exposed to TBBPA (0.75, 1.5 and 3uM), BPA (5,10, and 15uM) and TBBPA dimethyl ether (1,5,10uM). At 1.5 and 3uM TBBPA embryos exhibited 100% mortality before hatching. At 0.75uM, embryos exhibited edema, tail malformations and curvature to the spinal column. BPA exposure caused less severe phenotypes with only 15uM resulting in edema and blood pooling. TBBPA dimethyl ether treatment caused severe phenotypes but they differed from those seen with TBBPA treatment, which included hemorrhaging, prevention of hatching, and spinal curvature. These results illustrate the ability of indigenous microbes to O-methylate TBBPA in sediment samples as well as differing levels of toxicity of TBBPA and its metabolites resulting from these microbially mediated transformations.

307 BIOTRANSFORMATION OF 2, 3, 3, 3-TETRAFLUOROPROPENE IN RATS.

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2,3,3,3-Tetrafluoropropene (HFO-1234yf) is a non-ozone-depleting chlorofluorocarbon replacement with a low global warming potential and is developed as refrigerant. The biotransformation of HFO-1234yf was investigated after inhalation. Male Sprague Dawley rats were exposed to air containing 2,000, 10,000, or 50,000 ppm HFO-1234yf for 6 hours in a dynamic exposure chamber (n = 5/concentra-

tion). After the end of the exposure, animals were individually housed in metabolic cages and urines were collected at 6 or 12 hours intervals for 48h. For metabolite identification, urine samples were analyzed by 1H-coupled and decoupled 19F-NMR and by LC/MS-MS. Metabolites were identified by 19F-NMR chemical shifts, signal multiplicity, H-F coupling constants and by comparison with synthetic reference compounds. In all urine samples, the predominant metabolites were 2-acetylamino-3-(2,2,2-trifluoro-1-hydroxymethyl-ethylsulfanyl)propionic acid and 2-acetylamino-3-(3,3,3-trifluoro-2-hydroxy-propylsulfanyl)propionic acid (HFO-1234yf-MAs), whose signal intensity in 19F-NMR represented more then 85% (50,000 ppm) of total 19F related signals in the urine samples. 1,1,1-Trifluorolactic acid, 1,1,1-trifluoro-3-hydroxy acetone and trifluoroacetic acid were present as minor metabolites. Quantification of the HFO-1234yf-MAs isomers by LC/MS-MS showed that more then 90% were excreted within 18 h after the end of exposure (t1/2 app. 6 h). In rats, the mean recovery of HFO-1234yf-MAs excreted within 48 h in urine was determined as 0.297 ± 0.028, 0.627 ± 0.155, and 2.432 ± 0.864 micromoles at 2,000, 10,000 and 50,000 ppm, respectively suggesting only a low extent (< 1 % of dose received) of biotransformation of HFO-1234yf. The obtained results suggest that HFO-1234yf is subjected to a typical biotransformation reaction for haloolefins by cytochrome P450-catalyzed formation of 2,3,3,3-tetrafluoroepoxypropane at low rates, followed by glutathione conjugation or hydrolytic ring opening.

308 IDENTIFICATION OF A TRYPTANTHRIN METABOLITE IN RAT LIVER MICROSOMES BY LC-ESI MS.

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Tryptanthrin originally isolated from Isatis tinctoria L. has been characterized to have anti-inflammatory activities through the dual inhibition of cyclooxygenase-2 and 5-lipoxygenase mediated prostaglandin and leukotriene syntheses. To characterize phase I metabolite(s), tryptanthrin was incubated with rat liver microsomes in the presence of NADPH-generating system. One metabolite was identified by liquid chromatography/electrospray ionization-tandem mass spectrometry. M1 could be identified as a metabolite mono-hydroxylated on the aromatic ring of indole moiety from the MS^2 spectra of protonated tryptanthrin and M1. The structure of metabolite was confirmed as 8-hydroxytryptanthrin with a chemically synthesized authentic standard. The formation of M1 was NADPH-dependent and was inhibited by SKF-525A, a general CYP-inhibitor, indicating the cytochrome P450 (CYP)-mediated reaction. In addition, it was proposed that M1 might be formed by CYP 1A in rat liver microsomes from the experiments with enriched rat liver microsomes.

309 EFFECTS OF RUTAECARPINE ON PHARMACOKINETICS OF ACETAMINOPHEN IN RATS.

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Rutaecarpine, an alkaloid originally isolated from the unripe fruit of Evodia rutaecarpa, has been shown to be anti-inflammatory as it inhibits cyclooxygenase-2. It induces the activities of hepatic CYP 1A2, 2B, and 2E1 in rats. A possible interaction between rutaecarpine and acetaminophen (APAP) was investigated in male Sprague Dawley rats in the present study. When 25 mg/kg APAP was intravenously administered concurrently with 80 mg/kg rutaecarpine, the area under the curve of APAP in plasma was significantly decreased when compared to that of APAP alone. When the rats were pre-treated orally with 40 and 80 mg/kg rutaecarpine for 3 days, the % value of Cmax and area under the curve of APAP-sulfate conjugate were significantly decreased to 56.4% and 61.7% of the vehicle control group, respectively. These results suggest that rutaecarpine might cause changes in the pharmacokinetic parameters of APAP in rats.

310 METABOLISM OF FPP-3, AN ANTI-INFLAMMATORY PROPENONE COMPOUND, IN RATS.

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1-Furan-2-yl-3-pyridin-2-yl-propenone (FPP-3) is an anti-inflammatory agent with a propenone moiety. Following a single intravenous injection of male Sprague-Dawley rats with 4 mg/kg of FPP-3, three different metabolites of FPP-3 were identified as M1 (1-furan-2-yl-3-pyridin-2-yl-propan-1-one), M2 (1-furan-2-yl-3-pyridin-2-yl-propan-1-ol) and M3 (a glucuronide conjugate of M2) in rat urine by a liquid chromatography-electrospray tandem mass spectrometry. The structures of M1 and M2 were the same as observed previously following the incubation of rat liver microsomes with FPP-3 in the presence of NADPH. Although all metabolites of FPP-3 were identified in rat urine, only M1 and M2 were observed in the bile and feces. In addition, FPP-3 and its metabolites were mostly excreted into the urine. The M3 was identified as a glucuronide conjugate of M2 because of the addition of 176 Da from the protonated molecular ion of M2 in MS2 and because of the production of free M2 following an incubation of urine with β-glucuronidase. From these studies, a possible metabolic fate of FPP-3 could be proposed in vivo.

311 METABOLISM OF [^{14}C]CUMENE IN F344 RATS AND B6C3F1 MICE: FORMATION OF ALPHA-METHYLSTYRENE.

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Cumene (isopropylbenzene) is a high-production-volume chemical used in the manufacture of several chemicals (including phenol, acetone, acetophenone, and alpha-methylstyrene) and present in a variety of petroleum products. The US National Toxicology Program (NTP) has recently finished a 2-year toxicology and carcinogenicity study of cumene in F344 rats and B6C3F1 mice. As a support to the NTP study, the *in vivo* metabolism of [^{14}C]cumene was studied in male F344 rats and B6C3F1 mice of both sexes following oral and intravenous administration and the *in vitro* metabolism of [^{14}C]cumene was carried out in liver and lung microsomes prepared from female rats and female mice. The metabolite profiles in urine, bile, expired air, and microsomal incubations were determined by HPLC analysis. Urinary metabolites were isolated using HPLC and the structural identification was achieved with MS and NMR analysis as well as comparison with authentic standards. The expired air consisted of mostly cumene and up to 4% of alpha-methylstyrene. 2-Phenyl-2-propanol glucuronide, 2-phenyl-1-propanol glucuronide, and 2-phenyl-1,2-propanediol mono-glucuronide were identified in both urine and bile. The other metabolites present in the urine but not in the bile included 4-(2-hydroxy-2-propyl)phenylsulfate, 2-hydroxy-2-phenylpropylsulfate, 2-hydroxy-2-phenylpropionic acid, 2-phenylpropionylglycine, S-(2-hydroxy-2-phenylpropyl)-N-acetylcysteine, 2-phenyl-2-propanol, 2-phenyl-1-propanol, and 2-phenylpropionic acid. The microsomal incubation studies demonstrated that female mouse lung metabolized more cumene to 2-phenyl-2-propanol, 2-phenyl-1-propanol, and alpha-methylstyrene than female mouse liver or female rat lung and liver. [Supported by NIEHS Contract No. N01-ES-25483 and HHSN291200775562C]

312 SPECIFICITY OF RAT SULFOTRANSFERASES FOR HYDROXYLATED POLYCHLORINATED BIPHENYLS AND ITS MODIFICATION BY OXIDIZED GLUTATHIONE.

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Polychlorinated biphenyls (PCBs) are persistent environmental hazards to human health, and their hydroxylated metabolites (OHPCBs) may be biologically active. Reactions catalyzed by sulfotransferases (SULTs) in humans and other species are a potential metabolic route for OHPCBs, and inhibition of sulfation may also be involved in some toxicities. We hypothesized that OHPCBs are substrates and inhibitors of rSULT1A1 and rSULT2A3, major hepatic phenol and alcohol sulfotransferases, respectively, in the rat, a common animal model for in vivo studies on PCBs. Our second hypothesis is that regulation of rSULT1A1 by reduced and oxidized glutathione (GSH and GSSG) alters its specificity for OHPCBs. We investigated 15 congeners of OHPCBs (4-OH PCB 8, 14, 34, 36, and 78; 4'-OH PCB 3, 6, 9, 12, 25, 33, 35, 36, and 68; and 6'-OH PCB 35) as potential substrates and inhibitors of homogeneous recombinant rSULT1A1 and rSULT2A3. 4-OH PCB 8, 4'-OH PCB 3, 9, 12, 35, and 6'-OH PCB 35 were substrates for rSULT1A1, while the other OHPCBs were inhibitors. OHPCBs with two chlorine atoms ortho to the phenolic hydroxyl were the most potent inhibitors (IC50 values: 0.3-4.4 microM). None of the 15 OHPCBs were substrates for rSULT2A3, and 11 were weak inhibitors. After treatment of rSULT1A1 for 1 h at 25°C with 1 mM GSSG, 4'-OH PCB 6, 4-OH PCB 14, 4'-OH PCB 33, and 4'-OH PCB 36 were substrates for rSULT1A1, while they were not substrates of rSULT1A1 pretreated with 1 mM GSH. At 0.85 mM GSH: 0.15 mM GSSG, 4'-OH PCB 6 and 4'-OH PCB 33 were substrates, while 4-OH PCB 14 and 4'-OH PCB 36 were not. Thus, these SULTs exhibit differences in specificity for OHPCBs, and the specificity of rSULT1A1 for OHPCBs is altered by the oxidative environment of the enzyme, a finding of potential relevance to cellular oxidative stress. [Supported by NIH grant P42 ES013661]

313 DETERMINATION OF THE SITES OF HYDROLYSIS AND *IN VITRO* RATE CONSTANTS FOR METABOLIC AND NON-METABOLIC CLEARANCE MECHANISMS OF SULFURYL FLUORIDE IN THE RAT.

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Sulfuryl fluoride (SO_2F_2) is a structural and post-harvest fumigant used to control a wide variety insect pests. SO_2F_2 is a key alternative to methyl bromide, a stratospheric ozone depleting substance scheduled for phaseout by the Montreal Protocol. A preliminary physiologically based pharmacokinetic (PBPK) model was recently developed to quantitatively describe the metabolism and other processes that regulate the kinetic disposition of SO_2F_2 in blood and target tissues in the adult rat after inhalation exposure to SO_2F_2. This initial work indicated that hydrolysis is important in regulating tissue dosimetry of SO_2F_2; therefore, a series of in vitro studies were conducted in blood and selected tissue preparations to clarify the sites of hydrolysis and to obtain the rate constants for SO_2F_2 and fluorosulfate hydrolysis in these tissues. Rat whole blood, red blood cells (RBCs), plasma and rat liver and lung S-9 homogenates were incubated with 87 ppm SO_2F_2 for one hour. There was a greater than 80% decrease in SO_2F_2 levels that occurred in the presence of whole blood and the RBC fraction and a 50 % decrease in SO_2F_2 levels with the fresh plasma. In contrast to these results, there was no loss of the parent SO_2F_2 in lung or liver tissue sub-fractions at various substrate and protein concentrations. These findings will improve the accuracy of the model. Also, these findings will help to understand the relationship between internal dosimetry of SO_2F_2, fluorosulfate and fluoride at environmentally relevant concentrations and the toxic effects observed at high exposure concentrations.

314 REACTIONS OF SINGLET OXYGEN WITH FUROSEMIDE: POSSIBLE FORMATION OF REACTIVE ELECTROPHILIC INTERMEDIATES.

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In an effort to synthesize a secondary ozonide with potential antimalarial activity from a reaction between furosemide and singlet oxygen, derivative product(s) were made that may be useful in determining the toxic metabolites that result from the oxidation of furan-containing drugs *in vivo*. Typical experiments utilized furosemide (a diene; excess) and singlet oxygen (a dienophile) that was generated by slowly bubbling molecular oxygen through the reaction mixture that contained trace amounts of Rose Bengal as a photosensitizer, in quartz cuvettes. Reaction mixtures were irradiated at 254 nm using six side positioned UVC bulbs in a Luzchem photoreactor at room temperature for 15, 30, or 60 min. Proton NMR analyses of reaction mixtures irradiated for 15 and 30 min revealed the consumption of the furosemide. However, the growth of product signals was not consistent with the expected formation of a secondary ozonide(s) as predicted by ChemNMR H-1 Estimation, a function of ChemDraw Ultra. It is suggested that the desired Diels-Alder adduct, i.e., secondary ozonide, reacted via one or several reaction pathways to form 2-(4-carboxy-2-oxo-but-3-enylamino)-4-chloro-5-sulfamoyl-benzoate or 4-chloro-2-(2,5-dioxo-pent-3-enylamino)-5-sulfamoyl-benzoate. The formation of such electrophilic intermediates may add to our current understanding of the toxicity of furan-containing pharmaceuticals *in vivo*. [Funding support from NSF (grant number HRD 0450375) and NIH (grant number P20 RR 16456) is acknowledged; Corresponding author: michelle_claville@subr.edu.]

315 CHARACTERIZATION OF CYTOCHROME P450 ISOFORMS INVOLVED IN VINCLOZOLIN BIOTRANSFORMATION IN RAT LIVER.

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Vinclozolin (V) is a fungicide used in agricultural settings. V is hydrolyzed in vivo and in vitro to 2-[[(3,5-dichlorophenyl)-carbamoyl]oxy]-2-methyl-3-butenoic acid (M1) and 3',5'-dichloro-2-hydroxy-2-methylbut-3-enanilide (M2). V, M1 and M2 have antiandrogenic properties by interacting with the androgen receptor. There is no information about which cytochrome P450 (CYP) isoforms are involved in V biotransformation. Our objective was to determine rat liver CYP isoforms involved in V metabolism. V was incubated with non-treated adult male Wistar rat liver microsomes (1-2 mg) and 1 mM NADPH in 0.1 M phosphate buffer (PB) pH 7.4 and 5 mM $MgCl_2$, for 30 min at 37°C. V metabolites from incubation media were extracted in acetonitrile and analyzed by HPLC/DAD/MSD. Two metabolites were detected and identified as N-(2,3,4-trihydroxy-2-methyl-1-oxo)-3,5-dicholorophenyl-1-carbamic acid (M5) and [3-(3,5-dichlorophenyl)-5-(1,2-dihydroxyethan-methyl)-1,3-oxazolidine-2,4-dione] (M6). M6 was gradually converted to 3',5'-dichloro-2,3,4-trihydroxy-2-methylbutylanilide (M4) by incubation in PB pH 7.4 at 37°C ($t_{1/2}$=45 min) and both co-eluted in the same peak. The K_M for co-eluted M4/M6 was 226.5 μM and the V_{max} was 0.743 nmoles/min/mg protein. The K_M for M5 was 117.5 μM and the V_{max} was 0.231 nmoles/min/mg protein. M4/M6 and M5 formation was inhibited by pilocarpine (IC_{50}=7.2 and 22.6 μM, respectively), orphenadrine (IC_{50}=20.4 and 31.8 μM, repectively) and proadifen (IC_{50}=70.9 and 118.8 μM, respectively). Pilocarpine was a competitive inhibitor of M4/M6 metabolites with a K_i of 5.4 μM. The presence of anti-rat CYP2A, 2B1/2B2 and 3A2 antibodies in the incubation media strongly inhibited V metabolism. This inhibition ranged from 31 to 60% for M4/M6 and M5 formation. These results indicate that V is efficiently metabolized in rat liver at least by CYP2A, 2B and 3A subfamilies. V metabolism characterization will provide further insight into the relationship between toxicity and tissue dose of V and its metabolites (Project supported by Conacyt-Grant 45688-Q).

316 BASE-CATALYZED HYDROLYSIS OF ARTIMISININ TO A HYDROPEROXIDE DERIVATIVE: IMPLICATIONS TO MECHASIM OF ACTION OF ARTEMISININ AND ITS DERIVATIVES.

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Artemisinin (ART), a sequiterpene lactone endoperoxide isolated from *Artemisia annua L.* (Chinese herb), has long been used as anti-malarial agent. It is believed that the cleavage of the endoperoxide bridge by intra-parasitic heme, which results in the formation of O- and C-centered radicals, is crucial in causing the death of *Plasmodium falciparum*. This simplistic view of mechanism of action is however fraught with problems, particularly when an explanation is sought as to what makes such closely related derivatives as ART and dihydro-ART differ markedly in terms of their potency. In an attempt to understand the possible bioactivation through cleavage of the lactone-ring system, we examined the hydrolysis of ART as a function of pH (range: 7.0 to 12.0) and at varying initial concentrations of ART at room temperature. At pH ≥ 9.0, ART hydrolysed readily but with a small lag period. A raise in pH by one unit resulted in *ca* . 7-9-fold increase in the rate of hydrolysis, meaning that the reaction has an inverse first-order dependence on H^+. At a given pH, the rate of hydrolysis was found to be first order on ART. Reversed phase HPLC analysis on Discovery C18 column (4.6 x 100 mm, 5 μ particle size) showed a major peak, and the product thus isolated was found to be a peroxide. Unlike the parent coumpound ART, the hydrolysis product readily reacted in the Fe^{2+}/Xylenol Orange reaction. GC/MS analysis on RX1 column (60 m length, 0.25 mm id, 0.25 μ film thicknes; temperature programming: 105 °C - 5 min, 20 °C/min up to 280 °C, and 280 °C - 10 min)revealed a base peak at *m/z* 265. The product was identified (tentative) as 2-[2-formyl-2-hydroperoxy-4-methyl-3-(3-oxo-butyl)-cyclohexyl]-propionate. It is suggested that a similar cleavage by paroxonase and cellular esterases plays a role in the anti-malarial and cytotoxic actions of ART. [Funding support from NSF (grant number HRD 0450375) and NIH grant number (P20 RR 16456) to RMU is acknowledged; Corresponding author, e-mail: rao_uppu@subr.edu.]

317 RESVERATROL INDUCTION OF DRUG TRANSPORTER EXPRESSION IN MOUSE LIVER.

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Resveratrol (RES) is a plant phytoalexin with antioxidant properties, which is known to have beneficial effects in various tissues, including liver. Biotransformation enzymes and transporters play an important role in protecting an organism against toxic chemical exposure by mediating chemical absorption into liver, biotransformation, and biliary excretion. Several studies have demonstrated that RES treatment increases the expression of some biotransformation enzymes and transporters in cells, but few studies have examined the effect of RES treatment on biotransformation enzyme and transporter expression in vivo. Therefore, the purpose of this study was to determine whether administration of RES induces expression of various transporters in mouse liver. Mice were administered RES (100 mg/kg, bid, ip) for 4 and 14 days. Protein expression in liver crude membrane fractions was determined by Western Blot. Administration of RES for 4 days did not significantly change expression of Oatp1a1 1b2, or Mrp1-6 in liver. However, RES administration for 14 days did increase Organic Anion Transporting Polypeptide (Oatp) 1b2, as well as Multidrug resistance-associated proteins (MRP) 1-4 and 6. Treatment with RES increased Mrp1 2, and 4 protein expression levels in liver by at least 1.6 fold over that detected in vehicle-treated controls. Conversely, RES administration decreased Oatp1a1 and Breast cancer associated protein (Bcrp) in liver as compared to vehicle expression. This study demonstrates that RES administra-

tion is capable of modulating transporter expression in liver and could also affect hepatic uptake and excretory processes (This work was supported by RI-INBRE grant #P20RR016457 from NCCR, NIH)

318 HESPERETIN INDUCTION OF SULFOTRANSFERASES IN HEP G2 CELLS AND RAT TISSUES.

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Hesperetin is a flavonoid. It presents in high amounts in citrus fruits such as oranges, lemons, limes, and grapefruits. Hesperetin has a variety of biological and pharmacological activities including cholesterol-lowering, anti-influenza virus, antioxidation, and protective effects against cardiovascular diseases and cancers. Sulfotransferases are important phase II drug –metabolizing enzymes. They are involved in the regulation of hormones and detoxification of xenobiotics. In the present study, cultured human hepatic carcinoma cells (Hep G2) was used to evaluate hesperetin's effect on phenol-prefer sulfotransferase (hSULT1A1) and dehydroepiandrosterone sulfotransferase (hSULT2A1). The effects of hesperetin on the expression of aryl sulfotransferase IV (AST IV) and hydroxysteroid sulfotransferase (STa) in rat liver and adrenal gland were also investigated. Enzyme assay and western blot methods were used for protein level determination; reverse transcription-polymerase chain reaction and real time PCR were used for mRNA level determination. The result showed that the enzyme activity, protein (Western) and mRNA expression of both hSULT1A1 and hSULT2A1 were induced in HepG2 cells by hesperetin. The induction of both enzymes was dose dependent. The induction of hSULT2A1 was much higher than that of hSULT1A1. AST IV and STa in rat liver were induced after 7 days treatment with hesperetin. STa in rat adrenal gland were also induced. The induction of STa was much higher than that of AST IV. Our western blot results were in good agreement with PCR results, suggesting that the induction is in transcriptional level. These results suggest that hesperetin may be able to affect the bioavailability of endogenous hormones including dehydroepiandrosterone etc., and disrupt endocrine balance. Hesperetin may also affect xenobiotic detoxification and carcinogen bioactivation.

319 RECEPTOR-MEDIATED REGULATION OF HUMAN CYP450, PHASE II, AND TRANSPORTER GENE EXPRESSION BY SELECTIVE ACTIVATORS ASSESSED BY QUANTITATIVE RNASE PROTECTION ASSAYS.

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Phase I and II enzymes and transporter proteins are known to play important roles in the disposition of xenobiotics in humans and other model organisms. It is known that regulation of the expression of these toxicologically/pharmacologically-relevant genes can be governed by hormone nuclear receptors and other transcription factors (e.g. aryl hydrocarbon receptor (AhR)). In this study, cultures of primary human hepatocytes were used to assess the ability of selective nuclear receptor/transcription factor activators to modulate CYP450, Phase II, and transporter expression. Analysis of mRNA expression from primary human hepatocyte cultures treated for 48 hours was performed using a high throughput multiplexed RNAse protection methodology. These experiments were designed to examine the concentration-dependent effects of effectors, including PXR agonists (rifampicin and PB), CAR agonists (PB and CITCO), AhR agonists (3-MC, omeprazole, b-NF), a PPARa agonist (fenofibric acid), a FXR agonist (CDCA), along with other select receptor agonists (troglitazone, phenytoin, and cerivastatin), on the expression of 14 CYP450, Phase II, and transporter genes. In addition to the expected expression patterns elicited by prototypical inducers, several notable exceptions were observed including the concentration-dependent induction of CYP3A4 and suppression of BSEP (ABCB11) by fenofibric acid. In contrast, the FXR activator CDCA caused a marked induction of prototypical target gene BSEP yet caused a concomitant decrease in PPARa target gene HMGCS2. Overall these results demonstrate that a molecular-based strategy combining primary human hepatocyte cultures with multiplex analysis of receptor target gene expression profiles can provide a more effective tool to probe chemical-biological interactions.

320 POTENTIAL ROLES OF NF-KAPPAB AND NFE2L2 IN REGULATION OF HUMAN HEPATIC MRP4 GENE EXPRESSION.

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Development of tolerance to acetaminophen (APAP) hepatotoxicity has been documented in rodents and humans. This phenomenon is known as autoprotection and changes in the expression of hepatic drug transporter genes may contribute to this by efficiently removing the toxicant itself and/or mediators of oxidative stress generated during APAP-induced cell damage. Our previous studies revealed that hepatic multidrug resistance-associated protein 4 (Mrp4, Abcc4) is significantly induced in mouse following APAP exposure. Similarly, elevated levels of Mrp4 protein were observed in liver specimens from individuals that overdosed with APAP. Mrp4 induction in mice by APAP toxicity was dependent on activation of Kupffer cells, which produce many cytokines. Since NF-kappaB activation is the main converging point for several cytokine pathways, it is possible that NF-kappaB may be involved in transcriptional regulation of Mrp4 gene expression in mouse. Hepatic Mrp4 induction following APAP hepatotoxicity in mice is also dependent on nuclear factor (erythroid-derived 2)-like 2 (Nfe2l2), suggesting that Nfe2l2 may also be involved in the transcriptional regulation of Mrp4 gene expression in mouse. Based on the analysis of 10-kb upstream regulatory DNA sequence of MRP4 gene by TRANSFAC, 13 putative NF-kappaB binding sites were found. One of them was found at about -2000 bp, two groups of 2 were found at about -3800, -4500bp and one group of 5 was found between -7200~-8000bp. No obvious NFE2L2-binding sites were found. These results suggest that NF-kappaB might be directly involved in the transcriptional regulation of MRP4 gene expression while NFE2L2 may have an indirect role. We will further investigate the role of NF-kappaB and NFE2L2 in transcriptional regulation of human hepatic MRP4 gene expression by characterizing the interactions between NF-kappaB and the upstream regulatory region of MRP4 and between NFE2L2 and other transcription factors. This work was supported by National Institutes of Health Grant DK069557.

R 321 RISK ASSESSMENT FOR BIOTHERAPEUTICS.

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The evaluation of nonclinical safety of potential new drugs underpins the design and execution of their clinical development plans. Nonclinical safety evaluation for biotherapeutics is similar in principle to that for small molecules; however, the very nature of these molecules requires special considerations that are likely to be unique for each new entity. To make the nonclinical assessment even more challenging, newer biotherapeutics are becoming much more refined (species specific and potent) and diverse in nature. Additionally, with the merger of certain aspects of CBER into CDER, and in the aftermath of TeGenero and the report from Professor Duff's Expert Working Group, it is clear that regulatory expectations are changing. Specifically, at the writing of this abstract FDA is drafting a guidance document intended to supplement ICH S6 in the nonclinical assessment of biotherapeutics, EMEA has released draft guidance on the requirements to support first in human dosing (including a recommendation for dose setting in FIH studies), and ICH is considering whether to open the ICH S6 guidance for biologics for maintenance. These evolutions in science and regulatory policy present ever increasing challenges in the safety assessment of biotherapeutics. This roundtable will present information and raise questions for discussions related to increasing confidence in safety during biotherapeutics discovery, when and how surrogate models for safety assessment of biotherapeutics may be appropriate, how to use pharmacological data to help select appropriate doses for nonclinical safety assessment, and key considerations for dose selection in human studies.

R 322 THE FUTURE OF TOXICOLOGY.

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Toxicology benefits greatly from recent advances in biomedical sciences and technology that provide new opportunities to facilitate our understanding of the chemical and biological mechanisms of toxicity. However, the discipline is facing something of an identity crisis. Toxicology as a scientific discipline has traditionally combined fundamental and applied research. Unfortunately, during the past decade the balance has shifted in favor of applied research. This roundtable will consider the future directions in toxicological research and highlight some of the examples of how fundamental science can be used successfully to address applied issues of toxicological relevance. For example, cell death research can serve as an important link between basic science and toxicology and as an area of cross-fertilization between disciplines. Cell death is the result of toxicity, and analysis of signaling pathways is essential for verification of the targets and mechanisms of toxins action. Furthermore, novel molecular, biochemical, genetic and genomics approaches are increasingly used to understand the mechanisms of environmental toxicants. New data which combines knowledge of the toxicity mechanisms, gene expression profiling and the information on the ge-

netic diversity in the mammals makes it possible to determine what genetic variants correlate with susceptibility or resistance to toxicity and disease, thus potentially helping to identify a susceptible population for each exposure. In addition, uncovering the mechanisms by which agents cause adverse effects allows for a more refined assessment of dose-response, and therefore better predictions regarding thresholds for toxicity. Such information is critical for medical practice, drug development and setting environmental and occupational exposure limits. Finally, the public expects that the time frame from fundamental toxicological research to practical application must shorten commensurable with the urgency to deliver adequate risk assessment on issues relevant to public health and safety. Hence, tight interactions between fundamental and applied research is the future of toxicology.

323 THE UTILIZATION OF THE EPIOCULAR™ HUMAN TISSUE MODEL TO ASSESS AND COMPARE THE IRRITATION POTENTIAL OF MULTIPLE SURFACTANT SYSTEMS USED IN SHAMPOOS AND FACIAL CLEANSERS.

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Assuring the safety of cosmetics and personal care product through reliable in vitro assessment programs is an important goal. In addition, the Seventh Amendment to the Cosmetics Directive requires that after 2009, animal testing cannot be used to assess the eye or skin irritation potential of either cosmetics formulations or ingredients. In anticipation of this, we have developed a program to support the ocular safety evaluation of multiple surfactant systems used in shampoos and facial cleansers. This is particularly important as eye irritation is a likely occurrence in the use of these personal care products. The program relies on the results of a topical application of formulations to the surface of a three-dimensional, human cell-derived model of the corneal epithelium (EpiOcular™, MatTek Corp., Ashland, MA, USA) and monitoring time to toxicity. Over thirty different products formulated with a range of multiple surfactant systems, have been tested at dilutions of 2% and 10% to better reflect various normal exposure scenarios. The majority of the products had ET_{50}'s greater than 4 hours, as compared to the positive control (0.3% Triton X-100) which had an average ET_{50} of approximately 0.5 hours. We determined that the irritancy potential for most of the prototype shampoos fell in the mild to no irritation range. The effectiveness of the in vitro model has been assessed by comparing the in vitro results with consumer experience information.

324 COMPARISON OF THE EPIOCULAR ASSAY WITH HUMAN CLINICAL USE STUDIES AND POST-MARKET CONSUMER DATA.

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Several methods may be used to evaluate the potential for eye irritation in cosmetic products. Ten eye area cosmetic products were chosen for review of their in vitro and in vivo eye irritation studies. Post-market consumer feedback was also reviewed to provide additional data in assessing the suitability of the studies in predicting eye irritation potential. In the in vitro assay, formulations were applied neat to the EpiOcular tissue construct in order to determine the exposure time required to reduce the tissue viability to 50% (ET50). The human clinical studies were safety-in-use studies performed to evaluate eye irritation potential in contact lens wearers or individuals with self-perceived sensitive eyes. These studies are intended to evaluate "real-world use" under the supervision of an ophthalmologist. The ophthalmologic evaluation included a gross exam, slit-lamp exam and visual acuity exam. Post-market consumer feedback was compiled over eighteen months from consumer comments attributed to a specific product. All comments received were unconfirmed adverse reactions and not all were specific to eye irritation. The products had ET50 values ranging from >240 minutes to >1440 minutes and were all considered "non-irritating." All ophthalmologic examinations remained within normal limits during each study. A correlation was found between ET50 responses and eye-area irritation in the human clinical testing, indicating that the data from an EpiOcular may assay lend support to the results of an in vivo assessment of the product. The post-market data further support this observation. All unconfirmed consumer reactions were minor in nature, such as mild itching, and were consistent with minor events observed in the human clinical studies. The reactions commensurate with normal individual and/or seasonal phenomena and do not indicate significant product-induced irritation. Thus, in the products reviewed, the studies described accurately predicted low eye irritation potential.

325 THE DEVELOPMENT AND UTILIZATION OF AN *IN VITRO* SAFETY TESTING PROGRAM FOR HAIR CONDITIONERS.

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Assuring the safety of cosmetics and personal care products without testing in animals has long been the goal of many international companies. This concern has become even more important with the requirement of the Seventh Amendment to the Cosmetics Directive that after 2009 animal testing cannot be used to assess the eye or skin irritation potential of either cosmetics formulations or ingredients. To address this problem, the Alberto-Culver Company has developed a program to support the ocular safety evaluation of certain hair conditioners. This program relies on the results of a topical application of formulations to the surface of a three-dimensional, human cell-derived model of the corneal epithelium (EpiOcular™, MatTek Corp., Ashland, MA, USA) and monitoring time to toxicity (ET_{50}; MTT activity reduced to 50% of the control condition). Twenty-eight different formulations primarily based on either single or dual quaternary ammonium compound (quats) systems utilizing various combinations of seven different quats have been evaluated in the model. Potential safety of the materials was assessed by comparison to a benchmark material having a well established safety profile in commerce. Twenty-seven of the materials, including the benchmark, had ET_{50}'s of 24 hours or greater, indicating that they were quite mild. The effectiveness of the system has been assessed by comparing the in vitro results with consumer experience information.

326 EXPANDED UTILIZATION OF THE EPIOCULAR™ HUMAN CORNEAL TISSUE MODEL FOR OCULAR IRRITATION TESTING.

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The EpiOcular tissue model (OCL-200) is an organotypic model of the human corneal epithelium (HCE) cultured from normal human keratinocytes. Paraffin embedded, histological cross-sections show the structure of EpiOcular closely parallels that of the HCE. Since 1997, yearly average ET-50 (reduction of tissue viability to 50% after exposure to 0.3% Triton X-100) has ranged from 20.6 to 25.0 minutes with average CV (coefficients of variation) under 7%. Thus, EpiOcular has demonstrated long term reproducibility over the past 10 years.

Currently, the Draize test performed on rabbits is the predominant test used for the assessment of ocular irritation and for registering chemicals and cosmetics ingredients. However, the 7th amendment of the Cosmetics directive and REACH legislation will prohibit or severely restrict the use of animals for safety assessment of chemical substances used in cosmetic products or in general use.

EpiOcular has been increasingly used by many personal care and household product companies to determine the ocular irritancy of their products without using animals. In particular, EpiOcular has been very useful for evaluating water soluble and low irritancy materials. Currently, a new protocol to handle industrial chemicals spanning a range of chemical categories and irritancies has been developed. 110 materials (liquids and solids) including alcohols, hydrocarbons, amines, esters, and ketones have been evaluated. Using a direct MTT tissue viability protocol, a prediction model for discriminating between ocular irritants and non-irritants was developed. Testing performed on 76 liquid materials resulted in 94.4% sensitivity and 67.5% specificity. Testing of 30 solid materials resulted in 92% sensitivity. This new protocol developed will allow industry to comply with pending legislation and address multiple concerns including: i) consumer safety, ii) public concern for animal welfare, and iii) testing cost.

327 THE PORCINE CORNEAL OPACITY AND REVERSIBILITY ASSAY IS AN ALTERNATIVE ASSAY THAT PROVIDES EVIDENCE OF REVERSIBLE EYE IRRITANCY.

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MB Research is currently validating an ex vivo alternative assay that can assess both potential eye irritation and reversibility using an SOT Grant for Alternatives Research from Colgate-Palmolive. In the Porcine Corneal Opacity and Reversibility Assay (PorCORA), excised porcine corneas (byproducts from the meat industry) are cultured for up to 28 days. Corneas are treated topically with test substances and the resultant irritation to the corneal surface is measured by visualizing sodium fluorescein stain retention with UV light. The reversibility of the corneal damage is demonstrated by measuring the changes in retention of fluorescein stain in the same cornea over time in culture. As corneal re-epithelialization occurs, the area of fluorescein stain retention decreases. There has been favorable correlation between

recovery in the in vivo Draize rabbit eye irritation results (ECETOC, 1998 Eye Irritation: Reference Chemicals Data Bank) and the PorCORA for the chemicals tested (100% ethanol, 1% benzalkonium chloride, 3% sodium lauryl sulphate, 8% sodium hydroxide, phosphate buffered saline). In vivo data for this project will come from this data bank to avoid the use of more animals. Verification of the re-epithelialization process was done histologically as well as by confocal microscopy (see presentation by Piehl, Gilotti, Ball & Cerven). When present, damage to the corneas was visualized by fluorescein staining of the corneas prior to fixation. Re-epithelialization was demonstrated within 14 days for 100% ethanol, 21 days for 1% benzalkonium chloride, and 10 days for 3% sodium dodecyl sulfate. Corneas treated with 8% sodium hydroxide retained up to 21 days. On the rare occasion that corneas treated with phosphate buffered saline retained fluorescein, re-epithelialization was demonstrated within 3 days. Time course of re-epithelialization was demonstrated by histopathology of treated corneas from 1, 2, 3, 7, 14 and 21 days post treatment.

328 CONFOCAL MICROSCOPY OF REVERSIBILITY IN EXCISED PORCINE CORNEAS.

M. Piehl, A. C. Gilotti, A. Ball and D. R. Cerven. *MB Research Laboratories, Spinnerstown, PA.*

No established alternative ocular irritation assay can measure corneal tissue recovery and reversibility. With the support of a Colgate-Palmolive Grant for Alternative Research, MB Research has developed an alternative assay: Porcine Corneal Opacity Reversibility Assay (PorCORA). This assay measures corneal damage and recovery for extended time periods using excised porcine corneas. Test articles are dosed directly onto the corneal surface, and tissue damage is assessed by fluorescein retention in the same corneas over time. To confirm fluorescein retention results and characterize corneal recovery in the PorCORA system, cell viability was measured by confocal microscopy using a live/dead fluorescent staining kit. Cultured corneas were treated with either water or 3% SDS, then stained and observed via confocal microscopy on days 3, 6, and 10. On day 3, extensive cell death was observed in the SDS-treated corneas, but not water-treated corneas. By day 6, additional live epithelial cells were apparent on all corneas. At day 10 all corneas were populated with many live epithelial cells. Our results indicate that re-epithelialization occurred during the 10-day culture. These confocal microscopic observations support our hypothesis that corneal recovery and reversibility in the PorCORA culture system occurs, can be predicted by fluorescein retention, and can be correlated with Draize test results. One drawback of the fluorescent staining kit is that a cornea can only be imaged one time. An alternative imaging technique is reflective confocal microscopy, which uses inherent reflective properties of corneal tissue to characterize tissue damage to epithelial cell layers and deeper stromal keratocytes. Future work will include the use of reflective confocal microscopy to more accurately measure the extent of corneal injury at multiple time points during recovery in the same cornea.

329 AN INTER-LABORATORY STUDY OF SHORT TIME EXPOSURE(STE) TEST FOR PREDICTING EYE IRRITATION POTENTIAL OF COSMETIC INGREDIENTS AND FINAL PRODUCTS.

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Using SIRC (rabbit corneal cell line) cells, we developed an alternative eye irritation test: the Short Time Exposure (STE) test. This STE test is a cytotoxicity test using physiological saline or mineral oil as the test solvent. Evaluation exposure time is short (5 minutes), which is similar to actual exposure situations, and uses cell viability (CV) as the endpoint for irritation potential. In the STE test, two test concentrations (5% and 0.05%) are used to predict Draize test data obtained with 100% and 10% concentrations, respectively.
In the current study, the aim was to confirm the predictive capacity of the STE method for assessing eye irritation potential not only for ingredients but also for products. Furthermore, we evaluated the transferability and reproducibility of the STE methods in three laboratories. The inter-laboratory study was conducted using the same protocol, manufacturer's test chemicals, and cell lots.
Good transferability and reproducibility were obtained for both the ingredients and the products in all three laboratories. When compared to the Draize data, the STE 5% test dose data showed a good accuracy (about 80%) with the 100% test dose Draize data, and the STE 0.05% data showed a good accuracy (about 80%) with the Draize 10% data for all three laboratories. Our results provide a new prediction model using two test doses. We were also able to use the test doses for rank classification of ingredients and products. The three laboratories obtained identical rank classifications for the materials tested using our STE method. These data suggest that the STE method has a good predictive capacity, easy transferability, and good reproducibility, which would make the method a useful in vitro eye irritation test. In addition, the STE method is easy to perform and has the ability to evaluate eye irritation potential of both ingredients and products.

330 EVALUATION OF THE CHICKEN ENUCLEATED EYE TEST FOR ITS ABILITY TO PREDICT EYE IRRITATION POTENTIAL OF ZINC PYRITHIONE-BASED ANTI-DANDRUFF SHAMPOO FORMULAS.

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Evaluation of eye irritation of chemicals and formulations used in consumer and cosmetic products is important to ensure safety if inadvertent exposure to the eye occurs. Historically, we evaluated eye irritation of aqueous-based surfactant formulations using the in vitro Cytosensor test. Recently, Cytosensor predicted a substantial irritancy increase with reduced particle size zinc pyrithione (ZPT) anti-dandruff shampoo formulas that did not correlate with what was expected in terms of eye irritation based on historically available low volume in vivo rabbit eye studies or clinical eye instillation studies. In these experiments we evaluated whether an alternative eye irritation test method, the Chicken Enucleated Eye Test (CEET), can better predict ocular irritation of ZPT-based shampoos (as compared to the Cytosensor). A series of shampoo formulas with historically available human and/or historically available low volume in vivo rabbit eye data were tested in the CEET. These available eye irritancy data were compared to the results obtained in the CEET assay to determine how well this assay predicted the in vivo and clinical findings. Shampoo formulas were categorized as non- or slight irritants by slit lamp examination of corneal effects (i.e. swelling, opacity, fluorescein retention). Histopathological examination of treated corneas confirmed the findings made by slit-lamp examination. In general, CEET results correlated well with the historically available in vivo and clinical eye irritation data. The results obtained with the CEET methodology suggest that the CEET is a suitable and sensitive test to examine the eye irritation profile of ZPT-based AD shampoo formulas.

331 *IN VITRO* EVALUATION OF PHOTOREACTIONS IN THE SKIN USING EPIDERMAL SKIN TEST 1000 (EST-1000).

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Following ECVAM's recommendation to refine, reduce or replace animal testing, different suitable in vitro methods for the predictive identification of hazardous compounds have already been established. Using reconstructed skin (RS) as a test system provides fundamental advantages to single cell culture testing and leads to promising results as shown by different validation studies.
In 1998 the 3T3 Neutral Red Uptake Phototoxicity Assay developed by Spielmann et al. has been established as a suitable in vitro test method for the evaluation of risks derived from photoreactive compounds (OECD Test Guideline 432). However this test method works best with soluble and non-volatile chemicals. To assess other types of materials, we began to focus on the use of 3D skin models. In comparison to the murine 3T3 cell line these models provide several advantages. First of all human skin models are capable of reflecting topical exposure conditions followed by UV irradiation. Furthermore test methods based on tissue models are not limited to a rather small subset of chemicals since solid and insoluble test compounds can be applied very easily to the surface of an epidermal model. Additionally epidermal models like Epidermal Skin Test 1000 (EST-1000, CellSystems, Germany) are derived from Keratinocytes of human origin and therefore inter-species variation can be minimized.
In this study EST-1000 has been topically exposed to photocytotoxic compounds like chlorpromazine followed by defined UV irradiation. Depending on their concentration, the phototoxic potential of these compounds has been monitored. Additionally we demonstrated that the application of skin care products like sun blockers reduced the UV-dependent toxic effects of the test chemicals. We conclude that Epidermal Skin Test 1000 is a suitable in vitro tool for the evaluation of both, the phototoxic potential of chemicals as well as for the efficacy testing of products from cosmetic skin care products.

332 MICROFLUIDIC HEK CULTURES AS ALTERNATIVES TO MAMMALIAN MODELS.

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The ability to maintain microfluidic human epidermal keratinocyte (HEK) cultures opens the door to high throughput assessment of the toxicity potential of compounds without the use of animal models. A microfluidic HEK culture optimization study was conducted to determine conditions capable of maintaining healthy cells for 72 hours. A microfluidic culture device was developed which features a method to confine cell colonies to discretely patterned collagen shapes within the channels and a constant medium perfusion strategy to maintain healthy cells. We found that an initial cell seeding density of 15 to 20 percent confluency was required to grow a colony to 80 percent confluency in 72 hours. Colonies seeded below this range would never reach confluency and colonies seeded above this range would grow out beyond the bounds of the collagen patches. Over the span of perfusion rates studied cells proliferated well in the range of 0.2 to 0.8 microliters per minute through a culture channel containing approximately 600 cells. Once we identified the optimum culture parameters for HEK, we demonstrated the use of our microfluidic device in assaying the toxicity of ethanol. We seeded patches of HEK in a serial dilution microfluidic device which created a linear dilution of a stock ethanol solution. The 70% (v/v) ethanol was automatically diluted from 10%-90% of the original concentration and exposed to HEK cultures within microchannels. HEK exposed to ethanol dilutions of 10%-30% showed high viability while ethanol concentrations above 30% resulted in cell death on all HEK patches. These experiments show that microfluidic approaches can be used as alternatives to animal models and that they can be used as viable platforms for assessing the toxicity of chemical compounds.

333 PREDICTING SKIN IRRITATION: *IN VIVO* RABBIT TEST *VS.* 4H HUMAN PATCH TEST AND *IN VITRO* HUMAN SKIN MODEL TEST.

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Due to the Cosmetics Directive and the new EU regulatory framework REACH, replacement of the in vivo rabbit Draize test for skin irritation has become a priority. One key difficulty in determining the validity of any particular protocol/prediction model is that in vivo rabbit data are often both scarce and of limited utility for the prediction of the biological effects in man. In the current study, 25 materials were tested in a 4-hour human patch test (HPT) and two in vitro protocols utilizing the reconstructed epidermis model, EpiDerm. HPT and in vitro results were compared to the EU classifications for these materials which are based on rabbit data. Of the 15 rabbit irritants (EU classification), only 5 were found to be irritating to humans. Using the rabbit data to predict HPT irritancy resulted in a sensitivity of 100% and a specificity of 50%. Using the original EpiDerm test protocol evaluated in the ECVAM skin irritation validation study (15 min exposure/42h post-incubation) to predict HPT irritancy, 4 of 5 irritants were correctly identified (sensitivity 80%, specificity 75%). Using the modified EpiDerm skin irritation protocol (60min exposure/42h post-incubation), all chemicals irritating human skin were identified correctly. Consequently, existing animal data as well as human data should be taken into consideration to provide a final judgment about the predictive ability of a new alternative method. Unfortunately, in majority of cases, in vitro test protocols must be optimized to achieve concordance with historical animal data, excluding evidence of human response. However, in vitro models derived from human skin cells, if used in appropriate test designs, may prove to be more useful than the animal tests for human hazard assessment.

334 PERFORMANCE STANDARDS FOR THE USE OF *IN VITRO* TISSUES IN A REGULATED ENVIRONMENT.

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In vitro skin models are being proposed as alternatives to animal testing for safety evaluation of cosmetics, consumer products, and pharmaceuticals. Methods currently validated or in the validation process utilizing the EpiDerm skin model (EPI-200) include protocols for skin corrosion, irritation, and genotoxicity. For instance, EpiDerm skin irritation data utilizing the MTT assay resulted in 100% sensitivity and 60% specificity versus human patch test (HPT) results while rabbit tests gave 100% sensitivity and 50% specificity versus HPT results (n=25 materials). Likewise, data from the EpiDerm micronucleus assay for genotoxicity correctly predicted 13 genotoxins and 4 non-genotoxins. However, for these and other assays to be used in a regulated environment, it is important to know that the tissues models are reproducible both during and following the validation process (K. Gupta et al., Regul Toxicol Pharmacol. 2005 Dec;43(3):219-24). The current poster summarizes the long-term reproducibility and performance of EpiDerm (EPI-200) against benchmark chemical treatment. Quality control testing of weekly batches of EpiDerm was performed using the MTT assay. The exposure time needed to reduce the viability to 50% (ET-50) for 1% Triton X-100 was determined. The coefficients of variation (CV) for the negative control tissue (exposed to ultrapure H2O) have averaged below 7.5% for every year since 1997. In addition, the yearly average ET-50 values have ranged from 6.0 hrs to 7.5 hrs. A reproducible ET-50 is indicative of constant barrier properties which are important in determining the exposure of a chemical following dermal exposure. These results over the past 11 years of commercial production are designed to address regulatory concerns regarding performance standards over time.

335 EVALUATION OF EPIDERM FULL THICKNESS-300 (EFT-300) AS AN *IN VITRO* MODEL FOR SKIN IRRITATION.

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EpiDerm Full Thickness 300 cultures (EFT-300) consist of a highly differentiated skin model containing normal, human epidermal keratinocytes (NHEK) and normal, human dermal fibroblasts (NHFB). Dermal exposure of irritant chemicals is known to cause inflammation and cytokine expression in the skin. We evaluated the tissue engineered EFT-300 as a model to study skin irritation after exposure to irritant chemicals. PURPOSE: The objective of the present study was to systematically evaluate the EFT-300 as a human skin equivalent and to study the irritation potential of the aliphatic hydrocarbons, nonane, decane and dodecane. METHODS: Before treatment, irritant chemicals were mixed with equal volume of mineral oil (baby oil). The EFT-300 tissues were cultured in 6 well plates and topically exposed to 5ul of chemical mixture for 24 and 48hours. The MTT assay was performed to determine tissue viability. Histological sections of the EFT-300 were observed for any morphological changes for 24 and 48 hr. ELISA tests were performed to quantify cytokine and chemokine release including IL-1α, IL-6 and IL-8. TEWL and erythema scores of rat skin in-vivo were measured to find out the correlation between EFT cultures and rat skin. RESULTS: As a result of treatment with nonane, decane and dodecane, there was no reduction in tissue viability or any significant alteration in the morphology of EFT-300 cultures after 24 and 48 hours. As the chain length increased from C9 to C12, IL-1α and IL-8 release were found to increase in the order of dodecane>decane>nonane. This is in concordance with our earlier in-vivo studies, which demonstrated that as the chain length increased from C9 to C12, the TEWL and erythema increased proportionately. CONCLUSION: There is a significant correlation between in-vivo and in-vitro skin irritation studies, which suggest that EFT-300 can be used as an alternative to use of animals in skin irritation research.

336 DEVELOPMENT AND USE OF AN *IN VITRO* SAFETY TESTING PROGRAM FOR BATH AND BODY WASH PRODUCTS.

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Assuring the safety of cosmetics and personal care products without testing in animals is a goal common to many personal care products manufacturers, due both to ethical concerns for animal welfare, as well as concerns of the relevancy of the animal model to human responses. Alternatively, the use of human subjects to test the irritation potential of these products may be expensive, time consuming, and potentially controversial. To address these issues, we have developed an *in vitro* testing program to support the vaginal irritation safety evaluation of a variety of bath and shower cleanser products. Candidate formulations are diluted to 10% in water to model a maximum concentration expected in bath water, and the dilutions are applied topically onto the surface of a commercially-available three-dimensional, human cell-derived model of the vaginal epithelium for various exposure times. The time-to-toxicity for each candidate is determined as the exposure time which reduces relative viability to 50% of controls (ET_{50}). Over twenty-five liquid body cleanser formulations with similar surfactant systems but with different fragrances have been evaluated using the model. Test results were compared to those obtained from reference formulations assayed in parallel for which human clinical results were available. Since inception of the program in 2006, an intended progression to

milder formulations could be measured, as evidenced by an overall increase in ET_{50} values in candidate formulations (mean ET_{50} values for two projects in 2006 and one in 2007 were 1.24 h, 1.58 h and 2.33 h, respectively). The effectiveness of the testing program has been confirmed by the notably low, or non-existent frequency of consumer complaints regarding vaginal irritation during the critical six-month timeframes after the marketplace introductions.

337 *IN VITRO* MODEL WITH DISCRIMINATIVE POWER FOR SKIN SENSITIZERS BASED ON GENE EXPRESSION PROFILES OF HUMAN DENDRITIC CELLS.

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Up till now, predicting the skin sensitizing capacity of chemicals has been done by in vivo testing. To relieve the ethical and economical burden associated with animal tests, during the last decade the goal of many researchers and industries has been the development of an in vitro assay for classifying skin (non-)sensitizers. We approached this by a study of gene expression changes, induced in human CD34+ progenitor-derived dendritic cells (CD34-DC) from cord blood, by means of real-time RT-PCR. For a set of 21 chemicals, consisting of 10 known sensitizing and 11 known non-sensitizing compounds (listed below), we determined the expression profiles of 13 genes that were previously selected for their discriminating potential on the basis of extended microarray studies[1]. The relative fold-changes of the most discriminating genes were combined into one predictor variable using discriminant analysis. The predictive capacity of this new in vitro model was evaluated in a cross-validation and resulted in the correct identification of the sensitizing character of all 21 chemicals tested. These results suggest that our CD34-DC based classification model may be a valuable in vitro alternative to the LLNA for identifying the skin sensitizing character of chemicals. **Sensitizing chemicals**: dinitrobenzenesulfonic acid, dinitrochlorobenzene, dinitrofluorobenzene, p phenylenediamine, mercaptobenzothiazole, nickel sulphate, cinnemaldehyde, tetramethylthiuram disulfide, ammonium hexachloroplatinate IV and eugenol **Non-sensitizing chemicals**: dimethylsulfoxide, tributyltin chloride, triton X-100, benzalkonium chloride, phenol, L-ascorbic acid, L-glutamic acid, methyl salicylate, sodium dodecyl sulphate, p aminobenzoic acid, zinc sulphate [1]*Schoeters et al., Mol. Immunol. 2007, 44: 3222-33*

338 DEVELOPMENT OF AN ORAL PHOTO-LLNA TO IDENTIFY PHOTOALLERGENS.

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We previously modified a cytometry-based local lymph node assay (LLNA) to identify topically applied photoallergens, correctly classifying 13 photoallergens and non-photoallegens. Here we adapted the Photo-LLNA to determine the photoallergenic potential of orally administered compounds in the quinolone and quinoxalin chemical classes. CBA/J mice were administered test compounds by oral gavage for 3 consecutive days. Two sets of each group were dosed — one set irradiated (20 J/cm^2 UVA), and one set left unirradiated. On day 6, the mice were injected with BromodeoxyUridine (BrdU, i.p.), a thymidine analog that incorporates into the DNA of proliferating cells. Auricular lymph nodes (LNC) were removed and processed for flow cytometric analysis. The Stimulation Index (SI) was determined by measuring the total of number of proliferating LNC for each mouse, expressed as a ratio and compared to No-UVA or Vehicle controls. A positive response is indicated by a SI ≥3 for groups +UVA, with a SI <3 for unirradiated animals. Mice treated with olaquindox, enoxacin, and lomefloxacin all exhibited positive responses. Positive responses with significant ear swelling indicates possible (photo)irritation instead of (photo)allergenicity. As validated in our Flow Cytometry-based LLNA and dermal Photo-LLNA, additional endpoints were added to the Oral Photo-LLNA assay to help distinguish oral photoirritants from oral photoallergens. Olaquindox was tested in four separate assays, two of which resulted in significant ear swelling. In the last assay, immunophenotype markers B220+ (B cells) and CD3+ (T cells), and immune activation markers ($I\text{-}A^K$, CD69) were used as endpoints to differentiate sensitization and irritation. UVA treatment produced significant increases, compared to No-UVA and vehicle controls, in %B cells, B:T ratio, $\%I\text{-}A^K$+ cells and $\%I\text{-}A^K$/CD69+ cells, indicative of an allergenic response. Although more chemicals need to be tested, including a known photoirritant, this assay appears promising at identifying oral photoallergens.

339 EFFECT OF GROWTH FACTORS AND EXTRACELLULAR MATRIX COMPONENTS ON GENE EXPRESSION AND DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS.

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Mouse embryonic stem (mES) cells (ES-D3, ATCC) are pluripotent cells derived from the inner cell mass of mouse blastocysts. The cells are capable of forming embryoid bodes (EBs) as an intermediate stage of differentiation. *In vitro* culture of mES cells on culture inserts in the presence of growth factors (GFs) and extracellular matrix (ECM) components influences the formation of EBs and their differentiation toward specific lineages. We examined the effect of five GFs (EGF, KGF, amphiregulin, TGFβ1 and LIF) known to influence differentiation, on cells grown on filter inserts coated with ECM proteins (collagen types I, IV; fibronectin; laminin). Differential gene expression was detected using RT-PCR from mRNA purified from EBs grown between 1-16 days on inserts. Concurrently, transmonolayer electrical resistance (TMER) was measured as an indicator of tight junction formation. In addition, time-lapsed videos monitored morphological changes of cells in culture. Results suggest that undifferentiated mES cells and EBs preferentially express OCT-4 (transcription factor for undifferentiated cells and trophectoderm layers) and Afp (alpha fetoprotein), respectively. Differentiated cells from EBs show greater expression of Arnt (aryl hydrocarbon receptor nuclear translocator) and Egfr (epidermal growth factor receptor, both markers for differentiated cells), suggesting differentiation toward epithelial and/or epidermal lineages. EGF- (epidermal GF) and KGF- (keratinocyte GF) supplemented media on collagen–IV matrix induced most significant increase in TMER. The data supports previous paracellular permeability and acute cytotoxicity studies that GFs and ECM proteins induce formation of epithelial/epidermal-like membranes with increased TMER. Thus, the cell culture insert model represents a novel approach for inducing biological membrane formation for cytotoxicity testing and drug screening protocols. (Supported in part by grants from ARDF, 35336-985; IFER, 35262-985; NIH AREA, R15-ES012170-01).

340 IN VITRO ASSESSMENT OF THE SKIN IRRITATION POTENTIAL OF HAIR DYES WITH A RECONSTRUCTED HUMAN EPIDERMIS (RHE) MODEL BY VIABILITY MEASUREMENT (MTT) AND HISTOLOGY.

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In vitro methods using human epidermis models to assess the skin irritation potential of chemicals as a replacement of the Draize skin irritation test have been the subject of a recent ECVAM validation study. After pre-validation, these models were shown to be able to discriminate between irritants and non-irritants by means of a prediction model based on viability measurement in the MTT assay with a refined common protocol comprising a treatment period of 15 min, followed by a rinsing step and a post-exposure incubation of 42 h. In the present study, the applicability of this *in vitro* approach for hair dyes has been evaluated using the SkinEthic RHE model and hair dyes with different colouring and/or physicochemical properties. As hair dyes may interfere with MTT, histological evaluation of the tissues was performed as an additional endpoint. The materials were applied neat or diluted onto the surface of the epidermis cultures. The results demonstrate that MTT is suitable to differentiate between cytotoxic and non-cytotoxic hair dyes. Where appropriate, possible interference of the dyes with the test system was evaluated by including colour correction tissues and killed tissue controls. An overall good concordance was observed between cytotoxicity measured with MTT and histology. Most of these hair dyes were also tested for eye irritation with the same *in vitro* approach (MTT and histology), using a reconstructed human corneal epithelium model (SkinEthic HCE). In comparison, the MTT based *in vitro* epidermis model is suitable to investigate the skin irritation potential of hair dyes, whereas in the eye irritation model, the hair dyes were more likely to interfere with MTT by their intrinsic colour or by their reducing capacity.

341 HEALING OF DERMAL BURN WOUNDS IN THE EPIDERM-FT *IN VITRO* HUMAN SKIN MODEL.

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Dermal wound healing involves interactions between dermal fibroblasts and epidermal keratinocytes, as well as cell and extracellular matrix interactions. The current poster describes wound healing experiments conducted with a full-thickness in vitro human skin model (EpiDerm-FT). Normal human epidermal keratinocytes (KC) and dermal fibroblasts (FB) were cultured to produce the highly differentiated

full-thickness skin model. Histological examination of EpiDerm-FT shows a collagen dermis populated by viable FB and an epidermis of stratified KC including basal, spinous, granular and stratum corneum components. Small burn wounds of several mm in diameter were induced in the epithelial model by means of a battery operated cauterizer. The wounded EpiDerm-FT cultures were fixed at various time points and H&E stained paraffin sections were prepared to evaluate the wound and the wound healing process. Immediately after burn wounding, necrotic epithelium and denatured collagen dermal matrix were evident. Within one day, the denatured collagen matrix began to degrade and epithelial KC were observed migrating inward from the wound edges. Over a time course of seven days, migrating KC repopulated the wounded area to form a fully covered epithelium. Dermal fibroblasts were also observed to be proliferating within the wound area and generating new dermal matrix material. These results demonstrate that the EpiDerm-FT model may be useful for applications designed to elucidate dermal-epidermal interactions during wound healing and to evaluate the role of specific growth factors or new therapeutics in the dermal wound healing process.

342 HIGH THROUGHPUT, MULTI-PARAMETRIC MONITORING OF APOPTOSIS IN CELLS USING HIGH CONTENT SCREENING CELL BASED ASSAYS.

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Apoptosis is a common mechanism of cell death characterized by morphological changes including loss of mitochondrial membrane potential and nuclear condensation and fragmentation. Mitochondrial dependent apoptosis is accomplished by release of cytochrome c from mitochondria, activation of caspase 9, caspase 3, PARP cleavage followed by nuclear condensation and fragmentation. Apoptosis can also be triggered by activation of death receptors leading to activation of caspases 8 and 3 followed by nuclear condensation and fragmentation. High Content Screening (HCS) cell based imaging assays are quantitative and automated, enabling measurements of multiple targets in an individual cell. Using a panel of HCS cell-based assays for several of these apoptotic markers we were able to identify different pathways triggered by compounds, and we evaluated different DNA damage and oxidative stress causing agents to differentiate between different apoptotic pathways. We were able to simultaneously measure and correlate changes in both the nuclear morphology and apoptotic markers to differentiate between different pathways of apoptosis triggered by different compounds in the same cell. We evaluated time- and dose- dependent responses of these compounds and ranked them according to their ability to induce apoptosis. These multi-parametric measurements of nuclear morphology, mitochondrial changes and apoptotic markers using HCS demonstrated this as a useful and efficient platform to profile not only cell health but, also to give insights into the possible mechanisms of cell death.

343 AH RECEPTOR INTERACTION WITH E2F1 BLOCKS E2F1-INDUCED APOPTOSIS.

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Previously, we have shown that TCDD treatment of cultured hepatoma cells caused the recruitment of the AHR to E2F-dependent promoters and the concurrent displacement of the p300 histone acetyltransferase, suggesting that the activated Ah receptor disrupts chromatin remodeling processes associated with induction of E2F-dependent genes. In an extension of these studies, we have further investigated the role of AHR in cell cycle regulation by examining the impact of deletion of the retinoblastoma protein RB (p105) on the inhibition of cell cycle progression resulting from AHR activation. The rationale for these studies was that deletion or loss of RB heterozygosis is common during the carcinogenesis process. Our results show that AHR and E2F1 co-regulate a number of cell cycle-dependent genes and that AHR-E2F1 protein complexes suppress E2F1-mediated induction of pro-apoptotic genes and block apoptosis. In agreement with this observations, tissue-specific deletion of RB in the liver greatly increases the pro-apoptotic effect of AHR ablation. These results delineate a novel molecular mechanism whereby the AHR, a known transcriptional activator, modulates cell cycle progression and mediates S-phase-specific gene repression and inhibition of cell cycle progression when activated by xenobiotic ligands in normal cells, but displays antiapoptotic properties in cells lacking the retinoblastoma protein. The Ah receptor appears to act as an environmental checkpoint that senses exposure to environmental toxicants and responds by signalling cell cycle inhibition to prevent propagation of the genotoxic damage generated by toxic metabolites. Failure of other cellular checkpoints, as may happen during tumor progression, may reverse this AHR function and cause it to have a pro-proliferative effect. *Supported by NIH Grant R01 ES06273.*

344 1-NITROPYRENE (1-NP) INDUCES APOPTOSIS, NECROSIS AND PARAPTOTIC LIKE CELL DEATH IN HEPA1C1C7 CELLS.

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1-Nitropyrene (1-NP) is the predominant nitrated PAH emitted in diesel exhaust. Some of these nitro-PAHs including 1-NP have been found to be mutagenic and/or carcinogenic. Recently we have thus focused on the mechanisms of cell death induced by some nitro-PAHs and PAHs, which may have implications for their mutagenic and/or carcinogenic effects (Landvik et al Chem Biol Int, 167, 41-55. 2007, Holme et al Toxicology, 231, 1159-74, 2007). Here we present results showing that 1-NP induced apoptosis and necrosis in Hepa1c1c7 cells, a mouse hepatoma cell line. Interestingly, 1-NP also elicited paraptotic-like effects. Upon exposure to 1-NP, a dramatic increase in cytoplasmic vacuolization was observed already after 6 h exposure, and at 12 h the cells started to partly round up while still no apoptotic or necrotic cells could be seen. Electron microscopic examination revealed that the vacuolization was partly due to a dramatic mitochondria swelling. At 24 h a marked reduction in the rate of cell proliferation and both apoptotic cells as well as cells with plasma membrane damage were observed. At later time points (48 h) relatively higher number of cells had plasma membrane damage, but their chromatin were only partly condensed. Interestingly, the caspase inhibitor Z-VAD-FMK inhibited the formation of apoptotic cells, while cycloheximide reduced the apoptotic, necrotic as well as paraptotic like cell deaths. MAPKs seemed to be involved in these two processes as a marked activation was observed following exposure to 1-NP, and their inhibitors partly reduced the induced cell death. On the other hand, exposure to 1-aminopyrene (1-AP) only induced apoptotic and necrotic cell death to some extent, without the characteristic morphological changes seen with 1-NP.

345 1, 3-BUTADIENE INDUCED APOPTOSIS IN LNCAP CELLS IS MEDIATED IN PART BY REGULATION OF BCL2/BAX EXPRESION LEVELS.

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Animal and human population studies support the reproductive carcinogenicity of Butadiene and its metabolite BDO2. However,the toxicity and carcinogenicity of BDO2 on reproductive tissue such as the prostate or prostate cell culture has not been fully investigated. In the current study we determined whether BDO2-induced apoptosis involved modulation of Bcl2 (anti apoptotic protein) or Bax (pro apoptotic) expression levels at both the protein and mRNA levels. We subjected LNCaP cells to increasing concentrations of BDO2 (0.1nM to100nM) for periods of 6,12 and 24h. The effect of BDO2 was evaluated by measuring cellular cytotoxicity using MTT assay, morphological signs of apoptosis using Fluorescent microscopy, prostate cell integrity using PSA secretion by ELISA and gene expression of apoptotic proteins using Western blot analysis and QRT-PCR. The results of MTT assay indicate that BDO2 induced toxicity with GI50 greater than 100nM. Following 10nM exposure, PSA secretion was increased by 9% at 6hr, decreased by 32% at 12h and increased by 3.6% at 24h. Fluorescent microscopic examination revealed signs of BDO2-induced apoptosis at concentrations as low as 10nM at 6hr. Western blot analysis of Bcl2 and Bax expression revealed a 0.5-fold dose-dependent decrease as compared to control. On the other hand, mRNA expression of these proteins decreased 1-1.5-fold at BDO2 concentrations greater than 10nM. We also observed a corresponding increase in caspase levels which was both time and dose dependent. These results of our study suggest that, the cell death induced by BDO2 in LNCaP cells may be associated with alteration of three key cellular proteins Bcl2, Bax and Caspases in mediating cytotoxicity and may regulate cellular and molecular processes causing cell death by apoptosis.

346 ENHANCEMENT OF CYTOKINE-INDUCED APOPTOSIS BY BENZENE METABOLITES IS UNIQUE TO TNFα

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Chronic exposure to benzene can result in aplastic anemia, myelodysplastic syndrome, and, in some cases, acute myelogenous leukemia. The effects of chronic benzene exposure are exerted through its metabolites, which are known to directly induce apoptosis and alter proliferation in bone marrow stem and progenitor cells. In addition to direct activity, we have also shown benzene metabolites can alter cy-

tokine response, leaving cells susceptible to the pro-apoptotic effects of TNFα and enhancing the proliferative response to GM-CSF. Specifically, hydroquinone impacts proliferation pathways through activation of AP-1 and inhibits NFκB activation, leaving cells susceptible to TNFα-induced apoptosis. In order to further define signaling events impacted by benzene metabolites, we have treated erythroleukemic TF-1 cells with hydroquinone and various cytokines, and analyzed the effects of such co-treatment on apoptosis and signaling pathway activation. We show that IL-1β-induced activation of NFκB is inhibited by HQ, with no increase in induced apoptosis. In addition, the pro-apoptotic effects of LPS and IFNγ are not enhanced by HQ. Cells treated with cytokines with known anti-apoptotic effects, GM-CSF and SCF, do not prevent HQ and TNFα-induced apoptosis. We have previously demonstrated that HQ and other benzene metabolites directly target TNFα-induced IKK activation directly upstream of NFκB, and show here that this effect is specific for IKK; TNFα-induced phosphorylation and activation of JNK, P38MAPK, and p44/42MAPK are unaffected by HQ. These results further our understanding of altered cytokine response in cells treated with benzene metabolites by demonstrating specificity of the apoptotic response to TNFα and not other apoptosis-inducing cytokines, and show that several signaling pathways normally activated by TNFα are not inhibited by hydroquinone.

347 BREVETOXIN 2 ALTERS JURKAT E6-1 APOPTOSIS RELATED GENE EXPRESSION.

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Brevetoxins (PbTx) are cyclic polyether compounds produced by several types of marine dinoflagellates (including Karenia brevis and Chatonella antiqua) during red tide events in the Gulf of Mexico, off the coasts of Florida and North Carolina, and other coastal areas around the world. An estimated average of $18 million dollars is lost each year in the U.S. fishing industry due to red tides. Red tide blooms have been implicated in massive fish kills, bird deaths and marine mammal deaths. Necropsies of marine mammals killed by red tides in Florida have found inflammatory lesions in respiratory tissues with brevetoxin positive lymphocytes. Because of the immunological implications of brevetoxin exposure, we have chosen to use a human T cell lymphoma line to study the effects of brevetoxin. We have previously shown that Brevetoxin 2 10-6M causes Jurkat cell apoptosis at one and four-hour treatments as detected by flow cytometry. For this study, Jurkat E6-1 cells were exposed to brevetoxin 2 10-5M for four hours, RNA was then isolated and examined utilizing the Super Array platform to determine effect of brevetoxin treatment on apoptosis specific gene expression. The human apoptosis array includes the TNF ligands and their receptors; members of the bcl-2, caspase, IAP, TRAF, CARD, death domain, death effector domain, and CIDE families; as well as genes involved in the p53 and ATM pathways. Brevetoxin 2 treatment caused the down regulation of 31 genes in the Bcl-2 family, IAP family, CARD family, Caspase family, TNF receptor family, CIDE family, Death Domain family, TNF ligand family, and the p53 and DNA damage response family. Research supported by Marine Biotechnology in North Carolina.

348 TNF-α-MEDIATED DISRUPTION OF SPERMATOGENESIS IN RESPONSE TO SERTOLI CELL INJURY IS PARTIALLY REGULATED BY MMP-2.

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Mono-(2-ethylhexyl) phthalate (MEHP)-induced Sertoli cell injury is reported to induce germ cell apoptosis through the Fas/FasL signaling pathway. Our previous observations revealed that soluble TNF-α released by germ cells after MEHP exposure acts upon Sertoli cell TNFR-p55 and activates the NF-κB pathway, consequently resulting in a robust induction of FasL expression in Sertoli cells. MEHP-induced FasL expression was suppressed in TNFR-p55/p75 gene deficient mice, demonstrating the functional importance of TNF-α in FasL-mediated germ cell apoptosis. Metalloproteinases (MPs) are essential for processing the TNF-α precursor which is required for the action on TNFR-p55, and are regulated by the tissue inhibitors of metalloproteinases (TIMP) family. TIMP-2 mRNA and protein levels in the testis decreased early after MEHP exposure, and further investigations demonstrated that TIMP-2 was only expressed in primary Sertoli cells. The secretion of matrix metalloproteinase-2 (MMP-2) in primary Sertoli/germ cell co-cultures was induced at 3 h after MEHP exposure (1.95-fold) and its activity increased in a time-dependent manner as well. The addition of SB-3CT, a specific gelatinase inhibitor, decreased the activity of MMP-2 and significantly reduced soluble TNF-α production in the presence of MEHP. MEHP exposure caused an increase in soluble TNF-α levels (9.46-fold) in primary co-cultures, while the addition of recombinant MMP-2 protein only enhanced a 5.4-fold production of soluble TNF-α, indicating that MEHP-induced MMP-2 is, in part, responsible for the activation of TNF-α in the testis. Taken together, our observations indicate the important role of distinct MPs on the disruption of spermatogenesis in response to MEHP, and Sertoli cells control the sensitivity of germ cells to apoptosis by multiple mechanisms.

349 APOPTOSIS PRECEDES MULTI-ORGAN INFLAMMATION IN MRL+/+ MICE CHRONICALLY EXPOSED TO TCE.

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Trichloroethene (TCE) exposure, both environmentally and occupationally, has been implicated in the development of autoimmune diseases. The mechanisms by which TCE exposure leads to the development of autoimmunity are only partially understood. To further explore the events that lead to TCE-mediated autoimmunity, we treated female MRL+/+ mice with 0.5 mg/ml TCE in drinking water for 24, 36 and 48 weeks, and then fixed various tissues in buffered formalin. Staining of tissue sections with hematoxylin/eosin showed lymphocyte infiltration in liver, kidney, and lung after 36 weeks of TCE exposure which was more pronounced after 48 weeks of treatment. Apoptosis, measured by TUNEL assay, was found to be increased in liver, kidney, and lung after 24 weeks of TCE exposure, but did not change at 36 and 48 weeks. The increase in apoptosis observed at 24 weeks was accompanied by an increase in FasL expression as demonstrated by immunohistochemistry. The increase in apoptosis thus preceded the lymphocytic tissue infiltration. These findings suggest that increased apoptosis and probable delayed clearance of apoptotic bodies may participate in TCE-mediated autoimmunity, leading to a SLE-like disease. However, role of apoptosis and delayed clearance of apoptotic bodies in TCE-mediated autoimmunity remains to be established.

350 ETHANOL IS APOPTOTIC TO NEURAL CREST BECAUSE IT ABERRANTLY ACTIVATES SIGNALS THAT NORMALLY GOVERN NEURAL CREST APOPTOSIS.

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We have long observed that, in the chick embryo model, ethanol-induced apoptosis of neural crest coincides with the programmed deletion of neural crest from hindbrain segments (rhombomeres, r) 3 & 5; the explanation for this was unknown. Here we report that the signals initiated by ethanol within neural crest converge upon and activate those signals that normally induce their programmed cell death (PCD). Ethanol causes neural crest death through stimulation of Gαi2/Gβγ and subsequent Ca2+ mobilization thru a phosphoinositidyl phospholipase Cβ. Treatment of normal embryos with the intracellular Ca2+ chelator Bapta-AM also prevents the PCD within r3/5, as do antagonists of downstream mediators Calmodulin (calmidizolium) and CaMKII (myristolyted AIP, KN93). Following such treatment, ectopic neural crest cells emigrated from r3/5 and merged with existing neural crest migratory waves, demonstrating their rescue by the Ca2+ antagonists. As with ethanol, the Ca2+ signal used for PCD utilizes a phosphoinositidyl phospholipase Cβ, as U73122 & Xestospongin C pretreatment prevents r3/5 apoptosis. However, unlike ethanol, pertussis toxin had no effect on r3/5 PCD, nor did C3 exoenzyme. In contrast, NF449 wholly prevented endogenous cell death, suggesting that a Gαq accounted for the PIP2/PLCβ activity. In situ hybridization placed this endogenous Ca2+ activity downstream of events that determined hindbrain segmentation (e.g., normal expression of HoxA1, B1, & Krox20), and upstream of the r3/5 apoptosis signals (e.g., Bmp4 & Msx2 were repressed). These data suggest that ethanol is toxic to neural crest because it activates signals that converge upon those that govern the PCD in r3/5. To our knowledge this is the first mechanistic explanation for the long-standing observation (Menkes 1970; Sulik et al. 1988) that regions of toxicant-induced apoptosis frequently coincides with regions that normally undergo PCD. [Supported by NIH Award R37 AA11085]

351 MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN 1, MRP1, MEDIATES GLUTATHIONE RELEASE DURING APOPTOSIS.

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Intracellular glutathione (GSH) levels are tightly regulated at two levels: by regulating the rates of biosynthesis and of export from cells. The mechanism underlying GSH transport out of cells is not fully understood, but several members of the multidrug resistance-associated protein (MRP/ABCC) family have been identified as

potential candidates. A recent study provided evidence for a role of MRP1 in mediating both basal GSH release and the enhanced release of GSH that is observed in cells undergoing apoptosis (Hammond et al. J. Biol. Chem. 282:14337-14347, 2007). To test this hypothesis further, the present study characterized MRP1's role in basal and apoptotic GSH release using HEK293 cells stably transfected with human MRP1, or with MRP2, another member of the MRP family implicated in GSH export. The MRP1 overexpressing cells had significantly higher basal and apoptotic GSH release when induced with either Fas antibody or staurosporine. GSH synthesis rate in the HEK293-MRP1 cells was also markedly enhanced, and this higher GSH synthesis rate may have contributed to a lower level of apoptosis in these cells. Conversely, basal and apoptotic GSH release in the HEK293-MRP2 cells were similar to the vector-transfected cells, suggesting that MRP2 does not accept GSH on its own as a substrate. However, basal and apoptotic GSH release was enhanced with vincristine in the HEK293-MRP2 cells, indicating that MRP2 mediates GSH cotransport. Overall, these results suggest that MRP1 is a major contributor to both basal and apoptotic GSH release, whereas MRP2 is not involved in these processes. The enhanced GSH release and the concurrent decrease of intracellular GSH appear to be necessary for the progression of apoptosis. (Supported in part by NIH grants DK48823, ES01247, and ES07026).

352 P38 MAP KINASE MEDIATES APOPTOSIS THROUGH ACTIVATION OF FOXO3A AND INDUCTION OF BIM TRANSCRIPTION.

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Arsenicals are environmental toxicants. Sodium arsenite induces apoptosis in many different cell types and this apoptosis requires the stress-activated p38 MAP kinase and the pro-apoptotic BCL-2 family protein Bim. We demonstrated previously that p38 induces apoptosis by phosphorylating Bim at serine 65 and activating Bim's apoptotic activity. Here we report that sodium arsenite also stimulates both the transcription and protein expression of Bim. Ectopic activation of p38 is sufficient to stimulate Bim promoter, while blocking p38 signaling suppresses Bim induction. To identify mechanisms by which p38 regulates Bim expression, we examined if sodium arsenite activates FOXO3a, a member of the Fork head family of transcription factors, since Bim promoter contains two putative FOXO3a binding sites. Indeed, our data showed that FOXO3a translocates into the nucleus upon sodium arsenite treatment, indicative of its activation. Finally, RNAi knock down of FOXO3a inhibited Bim promoter activity, Bim protein expression, and apoptosis upon arsenite treatment. Our data reveal a novel apoptotic mechanism in which p38 induces apoptosis by activating FOXO3a which in turn induces the expression of Bim. Together with our previous study of post-translational regulation of Bim by p38, these results demonstrate that p38 induces apoptosis by regulating Bim two dimensionally at both the transcriptional and post-translational levels. Our data is also the first to report p38 as an upstream regulator of FOXO3a.

353 ARSENIC INDUCES DIFFERENT CELL SIGNALING PATHWAYS LEADING TO APOPTOSIS AND CELL CYCLE ARREST IN P53 +/+ AND P53-/- CELLS.

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The effects of arsenic on humans are well documented, but the mechanism of its perplexing paradoxical capability to act as both a carcinogen and a chemotherapeutic agent is unclear. The tumor suppressor gene p53 is crucial in maintaining genome integrity following exposure to environmental agents through the induction of cell cycle arrest, allowing for the DNA repair or apoptosis for cells with irreparable damage. In our previous study, we found As3+ uniquely caused toxicological changes in both genotypes, with more sensitivity in p53 knockout (-/-) cells illustrated by the dose-dependent increase in cytotoxicity and cell cycle alterations. Microarray-based gene expression analysis suggested that As3+ induced different pathways leading to cell cycle arrest and induction of apoptosis in the p53 wildtype (+/+) versus p53 -/- cells. In this study, we hypothesize that As3+-induced apoptosis in p53 deficient cells is through the activation of pro-apoptotic genes Noxa, and Puma. Cultures of p53 +/+ and p53 -/- mouse embryonic fibroblasts (MEFs) were treated with As3+, and cell viability and cytotoxicity were measured by Neutral red or LDH release assays. Total proteins were extracted and Western blot analyses of protein expression levels of Bcl-2 related proteins, including Puma and Noxa, were conducted. We observed differential dynamic changes of these proteins in p53 -/- as compared to p53 +/+ cells. This study suggests that cells with a functioning p53 protein utilize the pathway to mediate cellular defense and thereby mitigate damage. Alteration of p53-dependent cell cycle regulatory genes may play an important role in As3+ induced toxicity as well as carcinogenesis since most cells or tissues harbor the functional p53. Our current study also suggests that As3+ induces apoptotic process in p53 -/- MEF cells through the activation of p53-independent cell signaling pathway.

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354 TITANIUM DIOXIDE NANOPARTICLES INDUCE JB6 CELL APOPTOSIS THROUGH ACTIVATION OF THE CASPASE-8/BID PATHWAY.

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Titanium dioxide (TiO2) nanoparticles are manufactured worldwide in large quantities for use in a wide range of applications. Early studies have shown that the pathogenic effects of TiO2 depend on particle size. However, the molecular mechanisms involved in TiO2-induced cytotoxicity and carcinogenicity have not been clearly defined. The present study investigates TiO2 nanoparticle-induced cytotoxicity and the mechanism involved in this process in a mouse epidermal cell line, JB6 cells. For comparison, an equivalent concentration range of TiO2 fine particles was used in this study. We found that same mass of TiO2 nanoparticles exhibited stronger cytotoxity than that induced by TiO2 fine particles tested using the MTT assay. Moreover, a dual fluorescence dye assay indicated that TiO2 induced cell death through apoptosis in a time- and dose-dependent manner. Fluorescence staining using YO-PRO-1 or fluorescein isothiocyanate (FITC)–dextran dye demonstrated that TiO2 nanoparticles induced remarkable mitochondrial and lysosomal membrane injury. These results suggest the involvement of mitochondria and lysosomes in apoptosis induced by TiO2 nanoparticles. The signaling pathway involved in TiO2 particle- induced apoptosis was also investigated. Western-blot analysis showed an activation of caspase-8, caspase-3, Bid and Bax, and a decrease of Bcl-2, in JB6 cells treated with TiO2 particles. In conclusion, we have determined that caspase-8/Bid signaling may play a major role in TiO2–induced apoptosis and that mitochondrial and lysosomal injury may also be involved. Unraveling the complex mechanisms associated with these events may provide insights into TiO2-induced pathogenicity.

355 A COMPARATIVE EFFECT OF CADMIUM ON NON-TUMOR AND TUMOR DERIVED OSTEOBLASTIC CELLS.

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Cadmium is a known environmental and occupational toxin. There are multiple sources of cadmium, however, increasing discard of electronic products (e-waste) containing heavy metals makes cadmium exposure a growing public health concern. Humans are typically exposed to cadmium by consuming contaminated food or water, or smoking cigarettes. Exposure to cadmium is linked to bone diseases, including osteoporosis and osteomalacia. Previous studies in our lab have shown that cadmium induces apoptosis in the osteosarcoma cell line Saos-2 through a Caspase-3 dependent pathway. This study examined the effects of CdCl2 exposure on mouse non-tumor MC3T3-E1 osteoblastic cells compared to tumor derived Saos-2 cells. Our hypothesis is that non-tumor derived osteoblasts will react in a similar manner to tumor derived osteoblasts and undergo apoptosis when exposed to CdCl2. A cytotoxicity profile using the MTT assay was created for both cells lines exposed to 0-100 μM CdCl2 for 0-48 hours. An ApoPercentage kit was used to detect the translocation of phosphatidylserine to the outer leaflet of the cell membrane which is an indicator of apoptosis. The cytotoxicity profile indicated that MC3T3-E1 cells were more sensitive to CdCl2 exposure than Saos-2 cells, with EC50 values of 73 and 107 μM CdCl2 at 24 hours, respectively. Apoptotic induction was seen in both cell lines by 48 hours in response to 10 μM CdCl2 treatment. These results support our previous research demonstrating cadmium induced apoptosis in tumor derived osteoblasts and extend the work to also demonstrate cadmium induced apoptosis in non-tumor derived osteoblasts. Our long term goal is to develop an osteotoxicity model that will provide insight into how cadmium directly impacts bone and help in the creation of future treatments and prevention of bone diseases. Research funded by NIH-INBRE grant #P20RR016454.

356 LEAD INDUCES APOPTOSIS IN HUMAN LEUKEMIA (HL-60) CELLS *VIA* OXIDATIVE STRESS.

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Lead is a multi-targeted toxicant that affects many organ systems including; the gastrointestinal tract, hematopoietic system, cardiovascular system, central and peripheral nervous systems, immune system, and reproductive system. There are many published studies that have documented the adverse effects of lead in children and the adult population. Previous in vitro studies in our laboratory have shown that lead nitrate induces cytotoxicity to HepG2 cells in a dose-dependent manner.

However, the molecular mechanisms of toxicity and carcinogeneis are still largely unknown. In this research, we hypothesized that oxidative stress plays a key role in lead nitrate-induced toxicity and cell death in human liver carcinoma (HepG2) cells. To test this hypothesis, we performed lipid peroxidation assay to determine the cellular level of MDA production in HepG2 cells and the flow cytometric analysis of phosphatidylserine externalization to detect the percentage of death cell. Data generated from lipid peroxidation assay showed a significant increase ($p < 0.05$) in malondiadehyde (an end product of lipid peroxidation) production with increasing doses of lead nitrate. The results of flow cytometric assessment (Annexin V) also showed a strong dose-response relationship between lead nitrate exposure and early stage apoptosis in HepG2 cells. Upon 48 hrs of exposure, the results of annexin V/PI staining showed that the percentages of early apoptotic cells were 3%, 17%, 10%, 17%, and 80% in 0, 10, 20, 30, and 40 μg/mL of lead nitrate, respectively. The effect of lead nitrate was more powerful at 40 μg/mL ($P < 0.05$) compared to the control cells. In summary, these studies show that lead nitrate represents an apoptosis-inducing agent in human liver carcinoma (HepG2) cells. Findings from these studies indicate that cell death induced by lead nitrate is mediated through oxidative stress and phosphatidylserine externalization.

357 MECHANISMS OF CHEMICALLY-INDUCED DNA DAMAGE BY CHLORPYRIFOS IN THE ETIOLOGY OF INFANT LEUKEMIA.

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Maternal exposure to certain pesticides, pharmaceutical drugs and dietary agents has been associated with genetic abnormalities during fetal development and increased risk for infant acute leukemia (IAL) in offspring. While the mechanism linking contaminant exposure and disease is currently unknown, the majority of patients with IAL acquire 11q23 rearrangements of the mixed lineage leukemia gene (*MLL*$^+$) *in utero*. *MLL*$^+$ leukemias are rare in adults except in cases that arise subsequent to chemotherapy by topoisomerase II (topo II) inhibitors such as etoposide (VP-16). Alternatively, recent studies indicate that *MLL*$^+$ translocations may be induced by early chromatin fragmentation during apoptosis and are not specific to topo II. We have previously detected induction of *MLL*$^+$ events by VP-16 in human fetal liver CD34$^+$ hematopoietic stem cells (HSC). In this study, we report the occurrence of *MLL*$^+$ rearrangements in HSC after exposure to 10 μM chlorpyrifos (CPF) using fluorescence *in situ* hybridization. Compared to VP-16, CPF was not a topo II poison in *in vitro* reactions, nor did it stabilize topo II/DNA cleavage complex formation in CD34$^+$ HSC. However, both VP-16 and CPF induce early apoptosis in HSC as measured by high molecular weight DNA fragmentation and co-staining of TUNEL and active caspase-3 positive cells by flow cytometry. Furthermore, both CPF (0 – 100 μM) and VP-16 (0 – 10 μM) inhibited cell growth in a dose and time-dependent manner. These data suggest that HSC are susceptible to DNA damage by CPF, which may act as a potential leukemogen through apoptotic mechanisms. Future work will continue to identify the mechanism for chemically-induced *MLL*$^+$ translocations in HSC. Supported by NIH grants ES07032 and HD000836.

358 INHIBITION OF CA^{2+}-INDEPENDENT PHOSPHOLIPASE A_2-INDUCES P53-DEPENDENT PROSTATE CANCER CELL DEATH.

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This study tested the ability of the Ca^{2+}-independent phospholipase A_2 ($iPLA_2$) selective inhibitor bromoenol lactone (BEL, 0-10 μM) to induce cell death in p53-positive prostate cancer LNCaP cell lines and p53-negative prostate cancer PC-3 cell lines. Exposure of both cell types to BEL induced concentration and time-dependent decreases in the staining of 3-(4, dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT). Decreases in MTT staining were greater in LNCaP cells than PC-3 cells. Analysis of annexin V and propidium iodide staining using flow cytometery demonstrated that BEL induced apoptosis in LNCaP cells, but did not in PC-3 cells. Cell cycle analysis demonstrated that BEL induced a G1/G0-phase arrest in LNCaP cells, but had no effect on cell cycle in PC-3 cells. In addition, BEL treatment inhibited the ability of cisplatin to induce a S-phase arrest and vincristine to induce a G2/M-phase arrest. These data suggest the hypothesis that BEL induces prostate cancer cell death in a p53-dependent manner. In support of this hypothesis, treatment of LNCaP cells with BEL induced time- (0-24 hr) and concentration- (0 to 10 μM) dependent increases in the level of p53, compared to control cells. Further, BEL treatment increased the expression of p21 in a time-dependent manner. Increases in p21 occurred after increases in p53, however p53 increases were transient, while p21 levels were maintained up to 24 hr after initial exposures. BEL treatment did not increase p53 or p21 levels in PC-3 cells at any concentration or time points tested. Exposure of LNCaP cells to cisplatin increased p53 levels and pretreatment of LNCaP cells with BEL prior to cisplatin exposure decreased the level of p53 expression after 24 hr,, and unlike BEL, cisplatin induced increases in p53 were maintained., suggesting that these compounds induce p53 expression through different pathways. Collectively, these data suggest inhibition of $iPLA_2$ induces cell death in prostate cancer cells via a p53-dependent mechanism.

359 ROLE OF CALCIUM-INDEPENDENT PHOSPHOLIPASE A_2 IN SIGNAL TRANSDUCTION DURING CYTOSTASIS AND CELL DEATH IN PROSTATE CANCER CELLS.

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This study investigated the signal transduction pathways mediated by Ca^{2+}-independent phospholipase A_2 ($iPLA_2$) during cytostasis and cell death in prostate cancer cells. Treatment of LNCaP and PC-3 cells with the $iPLA_2$ selective inhibitor bromoenol lactone (BEL, 0-5 μM) induced time-dependent decreases in cell growth as determined by cell morphology, 3-(4,dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) and sulforhodamine B (SRB) staining assays. The effect of BEL on cell growth induced by lysophosphatidic acid (LPA: 0-10 μM) and EGF (0-1,000 ng/mL) was also assessed. LPA (10 μM) accelerated PC-3, but not LNCaP, cell growth at 24 and 48 hr, while EGF (1000 ng/mL) accelerated LNCaP, but not PC-3, cell growth at similar time points. Treatment of both cell types with BEL inhibited the mitogenic affects of LPA and EGF. These data suggested the hypothesis that an inhibitor of $iPLA_2$ decreases cell growth, possibly through the direct inhibition of mitogenic signaling pathways. In support of this hypothesis, treatment of cells with BEL decreased LPA- and EGF-induced phosphorylation of the EGF-receptor (EGFR), as well as LPA- and EGF-induced AKT and ERK phosphorylation. Further, BEL inhibited phosphorylation of EGFR by the matrix metalloproteinase (MMP) agonist *p*-aminophenylmercuric acetate. In contrast, BEL had no effect on LPA-induced inhibition of cAMP production, suggesting that $iPLA_2$ acts downstream of G-protein activation. Collectively, these data demonstrate that treatment of prostate cancer cells with an $iPLA_2$ inhibitor decreases cell growth in correlation with decreases in EGFR activation. The mechanisms appear to involve alterations in the activation of MMP. These data suggest a role for $iPLA_2$ in signal transduction and cytostasis in prostate cancer cells.

360 CHRONIC EXPOSURE OF CADMIUM RESULTS IN AN INCREASED CELL SURVIVAL AND ACQUISITION OF APOPTOTIC RESISTANCE OF TM3 CELLS.

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Cadmium is a naturally occurring bivalent metal. Cadmium and cadmium containing compounds are known carcinogens and can induce many types of cancer. Human exposure to environmental cadmium is a result of burning fossil fuels and municipal waste. Chronic toxicity from long-term exposure to cadmium in contaminated food and water has also been noted. The objective of this experiment was to study the effects of cadmium on the cell proliferation of testicular Leydig cells of mice (TM3-cells). Cells were placed in Dulbecco's modified Eagle Medium Nutrient mixture F-12; DMEM/F12 combined with 5% Horse Serum, 2.5% Fetal Bovine Serum. Cell cultures were treated to various cadmium concentrations for a short-term as well as a long-term period. The short-term batch was exposed to various concentrations of cadmium for 72 hours. Chronic exposed cells were tested in different concentrations of cadmium over a three month period. The resulting data for the short-term exposed cells showed that the high concentrations of cadmium (100ng/ml and 1 μg/ml) was toxic to the cells, where as the lower concentrations of cadmium (1 pg/ml, 100pg/ml, and 1 ng/m) showed no effect compared the control. In the chronic exposure cells, all concentrations exhibited an increased cell growth compared to the control indicating a resistance to apoptotic activity. Thus our study suggests; A.) Short term exposure to cadmium is toxic to TM3 cells B.) Chronic exposure of cadmium results in an increased cell survival and acquisition of apoptotic resistance of TM3 cells. Further research is needed to understand the mechanism for the acquisition of apoptotic resistance by TM3 cell with chronic exposure to cadmium.

361 ROLE OF CX32 IN STEADY-STATE HEMATOPOIESIS AND LEUKEMOGENESIS: STUDY IN THE CX32-KNOCKOUT MICE.

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The functional role of connexins (Cxs) in hematopoietic organs is poorly understood, except that the expression of Cx43 in bone marrow stromal cells sustains hematopoiesis. As Cxs are essential molecules for multicellular organisms, Cxs that

organize cell-cell communication within the hematopoietic progenitor cell compartment are surmised to be present in the hematopoietic tissue other than stromal cells. Recently, we have observed the functional impairment of hematopoiesis in Cx32-knockout (KO) mice. As results, the Cx32 molecule functioning in hematopoietic stem cell (HSC) compartment during steady-state hematopoiesis was observed for the first time; the expression of Cx32 at the level of mRNA determined by PCR analysis and Cx32 expression at the protein-level determined using an anti-Cx32 antibody were found only 28.0% of the $lin^{-}c\text{-}kit^{+}$ HSC-rich compartment, fractionated by a combination of immunobead-density gradient and immunomagnetic-bead separation, followed by flow-cytometric analysis. Furthermore, Cx32-KO mice showed increased leukemogenicity compared with wild-type mice after MNU injection; interestingly, in a competitive assay for leukemogenicity in mice that had been lethally irradiated and repopulated with a mixed population of bone marrow cells from Cx32-KO mice and wild-type mice, the resulting leukemias originated predominantly from Cx32-KO bone marrow cells. These studies imply that Cx32 play an essential role in tumor suppression.
In conclusion, the role of Cx32 in hematopoiesis was not previously recognized and Cx32 was expressed only in HSCs and their progenitors. We further conclude that the function of Cx32 in wild-type mice is to protect HSCs from chemical abrasion and consequent leukemogenic impacts.

362 EFFECT OF METHYLMERCURY ON NEURAL STEM CELLS DIFFERENTIATION DERIVED FROM CYNOMOLGUS MONKEY EMBRYONIC STEM CELLS.

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Neural stem cells (NSC) derived from monkey embryonic stem (ES) cells are useful to clarify molecular mechanisms underlying early neural developments in humans. In this study, we examined the effect of methylmercury (MeHg) on differentiation of NSC derived from the CMK-6 Cynomolgus monkey ES cell line to determine early neuronal toxicity of MeHg. Less than 1 μM MeHg exposure for 24 hr showed little cell toxicity in NSC, and the viability decreased to 20% in the presence of 10 μM MeHg. NSC for 14-days culture expressed both TuJ1 (neuronal marker) and GFAP (astrocyte marker) in immunocytochemistry. A real-time RT-PCR was performed to determine the effect of 0 - 1 μM MeHg on mRNA expression of several neuronal differentiation markers. PAX-6 expression was inhibited in the presence of 1 μM MeHg for 24 hr. These results would contribute to understanding molecular mechanisms for early neuronal toxicity of methymercury in humans.

363 BIOLOGICAL EFFECTS OF BENZO[A]PYRENE AND CHLORINATED BENZO[A]PYRENE IN MOUSE EMBRYONIC STEM CELLS.

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Polycyclic aromatic hydrocarbons (PAHs) are discharged from various emission source in air environment and in aqueous environment. It seems PAHs are received the changes in the aqueous environment by chemical reaction, photoreaction, metabolism of the organism and various treatment processes. Especially, in the case of raw water for drinking water source, PAHs are exposed chlorine in the process for the disinfection in drinking water treatment plant and then are reacted oxidation and/or chlorine substitution. It is known that chlorinated compounds cause the adverse effect on the human health. However, there is not so much information of the biological effect of chlorinated PAHs. It seems the tissues show the highest sensitivity and received serious damage for chemical compounds in the prenatal period. Therefore, we have examined the biological effects of benzo[a]pyrene (B[a]P) and chlorinated B[a]P (B[a]P-Cl) using mouse embryonic stem (mES) cells in differential state. The B[a]P-Cl was extracted from chlorine water. The B[a]P-Cl showed the cytotoxicity of high over 50 times in comparison with B[a]P in the undifferentiated mES cells. The maximum concentration that did not show the lethal effect was 0.2mg/L or less. The B[a]P-Cl showed the mutagenicity at 0.01mg/L concentration without metabolic activation by the umu test. B[a]P showed the mutagenicity at 0.04mg/L with metabolic activation and did not show the mutagenicity at 1mg/L or less without metabolic activation. These results indicated B[a]P increased slightly the mutagenicity by chlorination. In the established culture condition induced the differntiation, B[a]P-Cl was exposed at 0.005mg/L concentration for 12days from the differential start. In this condition, there were no remarkable effects on the expression level of GATA-4 gene that was the specific gene expressed for differentiation to primitive heart cells and on the morphological observation.

364 HUMAN EMBRYONIC STEM CELLS AS A MODEL OF DEVELOPMENTAL TOXICITY—A TOXICOGENOMICS APPROACH.

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Developmental toxicity is a major concern for new pharmaceuticals, and current testing uses a considerable number of laboratory animals. Human embryonic stem (hES) cells potentially offer a technology that may be used in the classification of candidate drugs in the early phase toxicity evaluation. Such a model would be time and cost efficient, as well as providing data from a cell state close to the developing human embryo. Altered gene expression may be predictive of teratogenic properties of a substance if important gene regulating pathways are affected, and may hence be used as appropriate endpoint in in vitro models. In the present study we explore the pluripotent hES cell line SA002 (Cellartis AB), using microarrays to profile the response to valproic acid (VPA), a known human teratogen causing e.g. spina bifida and cognitive disorders, and three closely related compounds with different potencies regarding in vivo teratogenicity in mice and histone deacetylase (HDAC) inhibition, a proposed teratogenic mechanism of VPA. hES cells in an undifferentiated state were exposed for 24 h to 1 mM VPA, 0.25 mM or 0.5 mM S-2-pentyl-4-pentynoic acid (more potent teratogen and HDAC inhibitor than VPA), 1 mM 3-propyl-heptanoic acid (potent teratogen but not an HDAC inhibitor), 1 mM 2-ethyl-4-methyl-pentanoic acid (potentially pharmacologically active non-teratogen and non-HDAC inhibitor), or 0.1% DMSO. Gene expression was subsequently profiled using Codelink Human Whole Genome BioArrays. We found the HDAC inhibitors to induce large and very similar changes for the most differentially expressed genes. Further, a concordance of altered gene ontology groups, predominantly neurogenic processes and microtubule depolymerisation, was evident between all the teratogenic substances. From these results it appears worthwhile to further investigate both SA002 as an in vitro model and gene profiling as a tool in developmental toxicity evaluation.

365 ARYL HYDROCARBON RECEPTOR ROLE IN HEMATOPOIETIC STEM CELL FUNCTION.

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The Aryl hydrocarbon Receptor (AhR) belongs to the basic helix–loop– helix (bHLH) Per-Arnt-Sim (PAS) superfamily of DNA binding proteins and it is selectively activated by dioxins. Exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) increases the incidence of hepatic tumors in animals and also has teratogenic effects. Increased incidence of leukemia and lymphoma has been reported in human populations accidentally exposed to dioxins. Although TCDD is known to alter bone marrow (BM) cells and function, the target cells and affected signaling pathways have not been identified. Following hematopoietic stress, normally quiescent hematopoietic stem cells (HSCs) undergo proliferation and differentiation. However, the molecular players that regulate this response of HSCs have yet to be identified. Here, we investigated the effects of TCDD-mediated AhR activation in essential cellular processes for HSCs such as the ability to reconstitute blood cell lineages and homing to BM. Mice were administered a single dose of TCDD (30 μg/kg) or vehicle. One week later we examined differential HSC behavior in functional studies. Reconstitution ability was studied using a competitive repopulation assay using irradiated CD45.1+ recipients and competitive donors. Additionally, a colony-forming unit-spleen assay was used to evaluate growth potential of HSCs and hematopoietic progenitor cells (HPCs). For evaluation of homing behavior, fluorescent-dye labeled donor cells were injected in irradiated mice. After 24 hours, homing to recipients' BM and spleen was analyzed. Our results show that activation of AhR decreases HSC's reconstitution ability as well as homing behavior while increasing growth potential of HPCs. The deregulation of these essential features of HSCs suggest that the AhR has a potential physiological role in maintaining homeostatic conditions in these and possibly other stem cells populations. Funded by NIH Grants ES01247 and ES04862.

366 DIFFERENTIAL INFLAMMATORY GENE EXPRESSION IN YOUNG AND AGED MICE DURING CHEMICAL INJURY-INDUCED HIPPOCAMPAL NEUROGENESIS.

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Adult neurogenesis occurs in the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG); however, the proliferative capability declines with age hindering repair and plasticity. In this study hippocampal neurogenesis was induced in

PND21 and 1 year-old CD-1 mice via intraperitoneal trimethyltin (TMT) injection. TMT injection induced damage and proliferation regardless of age. In the aged mice the degree of proliferation and differentiation into neurons, as detected by bromodeoxyuridine incorporation and NeuN localization, was significantly diminished compared with the young mice. Using laser-capture microscopy, SGZs were isolated from injured young and aged mice for molecular profiling by microarray analysis. A differential expression of 209 genes/ESTs occurred as a function of injury and age. Canonical pathway analysis (Pathway Architect®, Stratagene Inc) of microarray data identified expression of key molecules in the IL-6 signaling pathway (IL-6, Grb2, and RAF1) to be differentially induced with injury in the aged SGZ. Real-time PCR confirmed elevations of IL-6 and Grb2 in the aged SGZ following injury. Glia-derived neurogenic inhibitory factors have been previously reported in the adult brain, including interleukin 6 (IL-6). Increased IL-6 expression coupled with the lower level of proliferation observed in the TMT-injured aged mice suggests IL-6 contributes to inhibition of injury-induced neurogenesis with aging.

367 PERSISTENT ARYL HYDROCARBON RECEPTOR ACTIVATION RESULTS IN ALTERED NUMBERS AND FUNCTION OF HEMATOPOIETIC STEM/PROGENITOR CELLS.

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The aryl hydrocarbon receptor (AhR) is a ligand-activated cytosolic bHLH-PAS transcription factor whose endogenous function has not been identified. AhR activation by exogenous ligands has profound effects on the immune system and bone marrow populations, precise cellular and molecular mechanisms have yet to be determined. These studies were designed to test the hypothesis that alteration of marrow populations following persistent AhR activation is due to a primary effect on hematopoietic stem (HSC) and/or progenitor cells. A single dose of TCDD to mice resulted in an increase number of long-term and short-term HSCs as well as multipotent progenitor cells. These effects were not observed in Ahr null-allele mice. There was a time-dependent decease in B-lineage cells with a cocomitant increase in macrophage- and granulocyte-lineage populations. There was substantial increase in the CFU-Sd12 and functional multipotent progenitor cells by TCDD treatment. However, HSCs from TCDD-treated mice exhibited diminished capacity to reconstitute and home to the marrow of irradiated recipients. AhR was expressed in HSC and multipotent progenitor subsets. However, AhR mRNA was down regulated following treatment of animals with 5-fluorouracil.This result was consistent with the lack of response in bone marrow cells following the exposure of 5-fluorouracil-treated mice to TCDD. These findings along with the ability of TCDD to inhibit the growth of immature hematopoietic progenitor cells, but not more mature lineage-restricted progenitors, under in vitro conditions suggested that TCDD directly targets AhR contained in HSCs. These data are consistent with a hypothesis that the AhR has a physiological role in hematopoiesis and specifically in the regulation of primitive hematopoietic progenitor cells.

368 COMPARISON OF THREE *IN VITRO* STEM CELL HEMOTOXICITY, ATP-BASED, CYTOTOXIC ASSAYS FOR DRUG DISCOVERY AND DEVELOPMENT AND XENOBIOTIC TESTING.

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HALO (Hemotoxicity Assays via Luminescence Output) is a proliferation/cytotoxicity assay platform for lympho-hematopoietic cell populations that is based on the correlation of cellular proliferation status with intracellular ATP (iATP) concentrations. The release of iATP after incubation of the cells acts as a limiting substrate for a luciferin/luciferase reaction to produce bioluminescence which is measured in a plate luminometer. Calibration and standardization of the assay is performed using an external ATP standard curve. The present study involved a comparison of three different HALO formats all using fresh, primary human bone marrow target cells. The first format allowed the cells to grow under clonal conditions using a methylcellulose (MeC), semi-solid medium in a 96-well plate. The second format also utilized a 96-well plate, but the cells were cultured in the absence of methylcellulose using a suspension expansion culture (SEC). The last format was similar to the SEC, but used a 384-well plate format. Four compounds (AZT, cisplatin, mitomycin C and 5-fluorouracil) were initially tested on 7 lympho-hematopoietic populations (2 stem cell, HPP-SP, CFC-GEMM; 3 hematopoietic, (BFU-E, GM-CFC, Mk-CFC); 2 lymphopoietic, T-CFC, B-CFC). In the absence of compounds, a cell dose response for the CFC-GEMM stem cell population, demonstrated that the difference between formats showed that SEC was twice as sensitive than MeC and the 384-well plate format was twice as sensitive as the SEC. Furthermore, for a cell dose response from 500 to 25,000 cells/well, there was a direct correlation between the results from all three formats with a mean correlation coefficient (R) of 0.978, indicating that one assay format could be interchanged with another. When the 4 compounds were tested for cytotoxicity, there was little difference in inhibitory concentration (IC) values obtained using each of the 3 assay formats, indicating that different assay formats can be used with equal effectiveness at different stages of drug discovery and development.

369 THE ROLE OF MITOCHONDRIAL AND OXIDATIVE INJURY IN BDE 47 TOXICITY TO HUMAN FETAL LIVER HEMATOPOIETIC STEM CELLS.

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The polybrominated diphenyl ethers (PBDEs) are a group of flame retardants whose residues have markedly increased in the environment and in human tissues during the last decade. Of the various congeners, BDE 47 (2,2',4,4'-tetrabromodiphenyl ether) is typically the predominant congener observed in fish and wildlife samples, as well as in human tissues. Several studies indicate *in utero* transfer of PBDEs during pregnancy with residues accumulating in fetal tissues, and thus the potential for BDE 47 mediated injury *in utero* is of concern. In this study, we examined the mechanisms of BDE 47 mediated injury to primary human fetal liver hematopoietic stem cells (HSC), which comprise a large proportion of fetal hepatic cells and play a key role in hematopoiesis during fetal development. Incubation of fetal liver HSC with BDE 47 led to a loss of mitochondrial membrane potential and the onset of apoptosis. These effects were observed in the low micromolar range of BDE 47 exposures. At higher concentrations, BDE 47 elicited a loss of viability, which was accompanied by the generation of reactive oxygen species and peroxidation of HSC lipids. Preincubation of fetal liver HSC with *N*-acetylcysteine, a glutathione (GSH) precursor, caused an increase in cellular GSH concentrations, restored mitochondrial redox status, and ameliorated the toxicity of BDE 47. BDE 47 mediated cytotoxicity or oxidative injury was not evident at the lower concentrations (< 1 μM). Collectively, these data support a role for oxidative stress in the cytotoxicity of BDE 47 and indicate that oxidative stress-associated biomarkers may be useful in assessing the sublethal effects of BDE 47 toxicity in other models. However, the fact that BDE 47 undergoes a concentration-dependent accumulation in other primary cells in media that can underestimate cellular concentrations suggests that the HSC cell injury observed in our study may be of less relevance to human *in utero* PBDE exposures.

370 QUANTITATIVE MICROARRAY ANALYSIS BY "PERCELLOME" METHOD OF MURINE EMBRYONIC STEM CELLS AND EMBRYOID BODIES.

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Embryonic stem (ES) cells can differentiate into various cell types in cell aggregate form called embryoid bodies (EBs). EB serves as a most popular model to study molecular processes of early mammalian development. Here, we have undertaken a microarray gene expression analysis using Affymetrix GeneChip system on undifferentiated mouse ES cells (TT2) as day 0 and EBs from day 1 through day 7 with 0.5-day intervals. In this study, we have applied the quantitative microarray method (designated as "Percellome" method) for normalization, by which the data are expressed as mRNA copy numbers per cell. The so-called "stemness" gene in day 0 ES cells, such as Rex-1, Oct-3/4 etc. were gradually down modulation in differentiating EBs. Among various differentiation marker genes, mesodermal markers such as brachyury and goosecoid were transiently increased in EBs at day 3. This pattern was similar to that of the mouse embryo genes expression pattern. Time-dependent expression profiles of various nuclear receptors, including estrogen receptors, RXR, RAR, AhR, etc. in EBs were characterized. It is indicated that the ES/EBs Percellome database should provide a useful basis for the in vitro analysis system for the understanding of the molecular mechanisms of developmental toxicities induced by chemicals.

371 THE EFFECTS OF POLYCYCLIC AROMATIC HYDROCARBONS ON GAP JUNCTION FUNCTION IN AN ADULT HUMAN LIVER STEM CELL LINE.

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Gap junctional intercellular communication (GJIC) maintains tissue homeostasis and the blockage of GJIC by chemicals and other agents has been shown to be an underlying mechanism for tumor promotion and other pathologies. We investigated the effects of 1- and 2-methylanthracene (1-MeA, 2-MeA) on inhibition of

GJIC in a human liver stem cell line HL1-1. The stem cell nature of a clonally derived HL1-1 was indicated by the expression of α-fetal protein and vimentin, high proliferation potential, the ability of AIG and differentiation, and the lack of GJIC. When grown in a modified MEM, the cells exhibit high level of GJIC. The 1-MeA, which contains a bay-like region, inhibited in a dose and time dependent manner GJIC, while the 2-MeA, which contains no bay-like region, had no effect. Inhibition of GJIC by 1-MeA was dependent on activity of phosphatidylcholine-specific phospholipase C, and was also prevented by pretreatment with H89 (inhibitor of protein kinase A) or resveratrol (known antioxidant and anti-tumor compound). Inhibition of MAPK/ERK pathway did not affect 1-MeA-induced inhibition of GJIC. These results are in an agreement with our previous observations from rat liver epithelial oval (stem)-like cells WB-F344, and indicates involvement of similar signal pathways in response to 1-MeA in both cell lines. Support: NIEHS grant #R01 ES013268-01A2 to BLU.

372 AHR PATHWAY GENES ARE EXPRESSED IN HUMAN EMBRYONIC STEM CELLS.

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The use of human embryonic stem (hES) cells is an important advance in toxicity testing and drug development, yet only two percent of all published reports in hES cells study toxicological endpoints or pathways. The pluripotent nature and unlimited proliferative ability of hES cells afford numerous advantages for toxicological testing including the potential for renewable, more consistent cell sourcing for difficult to obtain human organs as well as an alternative to costly animal studies, which have historically been complicated by interspecies differences.
The aryl hydrocarbon receptor (AhR)/aryl hydrocarbon receptor nuclear translocator (Arnt) pathway is responsible for the metabolism of various xenobiotics such as polycyclic aromatic hydrocarbon (PAHs). To date, no studies have been published demonstrating the presence of AhR, Arnt or other pathway members in hES cells. 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) is a potent environmental contaminant known to cause a broad spectrum of toxic effects through activation of the AhR/Arnt pathway. Through quantitative PCR analysis, we have found that AhR and its dimerization partner, Arnt, are expressed in both hES cells and human embryoid bodies (hEBs) at stages of increasing differentiation. Following TCDD exposure, hES cells and hEBs do not show visible differences in cellular morphology or differentiation. Finally, TCDD treatment appears to cause an induction in *CYP1A1* mRNA expression in both hES cells hEBs, which introduces the possibility that CYP enzymes are functional early in development. These findings may further support the use of hES cells as sensitive predictors of toxicity and teratogenicity.

373 THE MECHANISM OF GALACTOSAMINE TOXICITY AND IDIOSYNCRATIC RESPONSE; A METABONOMIC STUDY.

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Galactosamine (galN) is an example of a classic 'model' hepatotoxin that has been widely used in experimental studies on liver damage, however the mechanism of galN-induced toxicity has not yet been fully resolved. Inter-animal differential sensitivity to the compound is common with clear responder and non-responder phenotypes apparent post-galN dosing, responders presenting with differential degrees of liver necrosis whereas non-responders exhibit no toxic response. A cohort of animals were treated with galN which resulted in the attainment of a sub-set (25 %) of animals that presented with no toxic lesion and were classified as non-responders, as determined from clinical chemistry and histopathological analyses. Metabonomic data from urine, serum and faecal extracts were generated using high-field solution state 1H NMR and of liver using magic angle spinning NMR. The urine NMR spectra of responders revealed increased levels of galN at 8h and of glucosamine and N-acetylglucosamine at 24h relative to controls and non-responders. The serum of responders had elevated levels of tyrosine, betaine, lactate and D-3-hydroxybutyrate. The liver MAS NMR spectra of responders revealed high levels of UDP-N-acetylgalactosamine, UDP-N-acetylglucosamine, lipid triglycerides and tyrosine. Furthermore, the faeces of responders had higher levels of both branched chain and aromatic amino acids. This approach enabled a comprehensive determination of the metabolic differences between responders and non-responders in all sample matrices which provided a systems level view of idiosyncratic response to galN toxicity and enabled us to move towards elucidating a mechanism for idiosyncratic response. This study was undertaken as part of COMET 2, which aims to utilize metabonomics as a 'top-down' systems biology driver to direct research and experiments in the determination of mechanisms of toxicity.

374 PHENOTYPIC ANCHORING OF GENE EXPRESSION DATA FROM ACETAMINOPHEN HEPATOTOXICITY STUDIES IN THE MOUSE MODEL OF THE HUMAN POPULATION REVEALS BIOMARKERS OF RESPONSE.

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Traditional toxicogenomic investigations of drug- or chemical-induced toxicity usually do not consider the potential effect of the genetic make-up of the model organism on the progression of chemically-induced adverse events. Our previous studies showed that a genetically diverse panel of mouse inbred strains, referred to as a "mouse model of the human population" (MMHP), can be useful as an animal model to understand and predict acetaminophen (APAP)-induced liver toxicity in a genetically heterogeneous human population. In this study, we hypothesized that changes in gene expression in liver, when correlated with an observed toxicity in a large panel of inbred mouse strains, will reveal biomarkers of effect that may be better reflective of population-wide responses. In this study, 36 inbred mouse strains were administered an acute dose (300 mg/kg) of acetaminophen (N=4) or vehicle (N=2) and RNA was extracted from left liver lobes at 24 hr post-dosing (*i.g.*). Analysis of differential gene expression based upon the biological replicates within strain, the treatment, and the sample liver necrosis score yielded several robust biomarkers of response that include *oncostatin M receptor*, a member of the IL-6 family of cytokines, and *interleukin 6 signal transducer*, a gene shown to be involved in acute phase gene expression that provides hepatocellular protection following lipopolysaccharide treatment. Interestingly, we also determined that genes previously not known to be modulated in APAP-induced hepatotoxicity show treatment-induced transcript changes that are correlative with liver necrosis; these include *peroxisome biogenesis factor 1* and *importin 4*. Taken together, the data suggest that the MMHP may have wide applicability for the phenotypic anchoring of xenobiotic-induced hepatotoxicity and toxicogenomic endpoints.

375 EVALUATION OF INDIVIDUAL BILE ACIDS AS BIOMARKERS OF HEPATOTOXICITY.

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Increases in total circulating bile acid concentrations have been utilized as indicators of hepatobiliary diseases. These measurements do not portray the dynamic changes of the individual bile acids, yet knowing how changes in the individual concentrations correlate to hepatotoxicity may help to understand the underlying mechanisms of toxicity and/or establish a biomarker for a particular disease state. Earlier and more specific biomarkers of hepatotoxicity would provide added value to pre-clinical studies by enabling better prediction of hepatotoxic events. Serum samples from rats dosed with known hepatotoxicants were analyzed by a LC/MS-based, non-targeted metabonomics approach and a LC/MS quantitative assay for individual bile acids. Metabonomics data identified those metabolites that changed in relation to hepatotoxic events, while the LC/MS assay determined the response of 13 individual bile acids to those events. Results were evaluated against histopathology findings, clinical chemistry parameters, and other proposed biomarkers. Unsupervised principal components analysis of the metabonomics data indicated that cholic acid (CA) and chenodeoxycholic acid (CDCA) were significantly up regulated in those animals dosed with hepatotoxicants when compared with control animals. In the LC/MS assay, the concentration of four bile acids, including CA and CDCA, were observed to increase significantly based on fold change and p-value. Some individual bile acids better predicted the hepatotoxic potential of a compound in comparison to any other clinical chemistry parameter. Additional serum samples from other investigative studies were analyzed. Changes in individual bile acid concentrations were observed, some of which were similar to previous findings. The differences observed may be related to the type of liver injury. Additional studies are required for the biological validation of bile acids as biomarkers of hepatotoxicity in pre-clinical studies, as well as determining their relationships to specific disease states.

376 DETERMINATION OF BIOMARKER GENES FOR THE EXPOSURE OF ARISTOLOCHIC ACID IN RAT KIDNEY.

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Aristolochic acid (AA) is the active component of herbal drugs derived from Aristolochia species that have been used for medicinal purposes since antiquity. AA induced nephropathy and urothelial cancer in humans and malignant tumors in the kidney of rodents. Although our previous study found that AA induced more

than two thousand differentially expressed genes in rat kidney, a reasonable number of signature genes for the AA exposure have not been determined. In this study, we explore the selection methods for multigene predictors for AA exposure. Lists of differentially expressed genes for AA treatment and riddelliine treatment (as a control for testing the specificity of the AA predictors) were generated from different microarray platforms: Affymetrix (Rat Genome 230 2.0), Applied Biosystems (Rat Genome Survey Microarray), GE Healthcare (Rat Whole Genome Bioarray,300031) and Agilent (Whole Rat Genome Oligo Microarray, G4131A, used for generating testing data). Affymetrix was performed at two independent test sites, one for building the predictors and the other for generating testing data. A set of genes were selected by fold-change ranking when $p < 0.0001$ from the common genes among Affymetrix, Applied Biosystems and GE Healthcare and from each individual platform with or without biological function filtration. The sensitivity and specificity of these predictors were determined by evaluating their predictive percentages for the testing data. We found that the biomarker genes from cross-platform comparison were more accurate for predicting the AA exposure than those from an individual platform; and that the biological function selection increased the specificity of the biomarker genes. Our results suggest that such an integrated analysis promises to be a valuable method for generating biomarker genes.

377 BIOMARKER DOSE RESPONSE PROFILING TO THE NEPHROTOXIN d-SERINE IN FISHER 344 RATS.

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An examination of current literature revealed several biomarkers predictive of acute, chronic, chemically-induced, or disease-induced kidney damage. We examined the protein expression profile in urine of each marker in a time course response using several doses of the known nephrotoxin d-serine. Male Fisher 344 rats were dosed with 5, 20, 50, 200, or 500 mg/kg d-serine in saline, whereas control animals were dosed with saline alone. Urine was collected from animals prior to dosing and at 24, 48, 72, and 96 hours post-dosing and frozen within 1 hour of end point collection. The samples were assayed by ELISA for clusterin, retinol binding protein 4 (RBP4), heme oxygenase 1 (HO-1), osteopontin (OPN), Yb1 (mu) glutathione S-transferase (GST), β2-microglobulin, and renal papillary antigen 1 (RPA1). In addition, the well-characterized kidney markers neutrophil gelatinase associated lipocalin (NGAL) and kidney injury molecule-1 (Kim-1) were assayed as indicative comparison markers. Biomarker levels remained constant for control animals and in animals dosed with 5, 20, and 50 mg/kg d-serine throughout the time course. However, for animals dosed with 200 and 500 mg/kg d-serine, significant increases were observed with peaks at either 24 hours post-dosing (clusterin, RBP4, HO-1, Yb1 GST, and β2-microglobulin) or 48 hours post-dosing (OPN and RPA1). HO-1 and Yb1 GST levels dropped to pre-dosing levels at 48 hours post-dosing, and clusterin, RBP4, OPN, β2-microglobulin, and RPA1 levels dropped at 72 hours post-dosing. However, marked increases in clusterin, OPN, and RPA1 levels were again observed at 96 hours post-dosing. Along with the existing journal based data, these expression profiles in response to a kidney toxin indicate that the examined proteins differ in the optimum response time and dosage, and are more "broad based" biomarkers of kidney damage rather than indicative of a specific acute, chronic or disease kidney state.

378 KIDNEY INJURY MOLECULE 1 (KIM-1) AS AN EARLY BIOMARKER OF CADMIUM NEPHROTOXICITY: COMPARISON WITH URINARY CADMIUM AND ALPHA-GLUTATHIONE-S-TRANSFERASE (ALPHA-GST).

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As a result of the widespread use of Cd in industry and its extensive dissemination in the environment, there has been considerable interest in the development of early biomarkers of Cd-induced renal injury. Kim-1 is a transmembrane glycoprotein that is normally not detectable in kidney, but is up-regulated and shed into the urine following ischemic or nephrotoxic injury. Recent studies utilizing a subchronic model of Cd exposure in the rat have shown that Kim-1 is a very early urinary marker of Cd-induced renal injury; urinary levels of Kim-1 were found to be elevated 3-4 weeks before the appearance of metallothionein and Clara cell protein-16, which are classic traditional makers of Cd toxicity. In the studies described here, we have compared the time course for the appearance of Kim-1 in urine with the time course for the appearance of Cd, itself, and alpha-GST, both of which have recently been proposed as possible markers of Cd nephrotoxicity. Male Sprague-Dawley rats weighing 250-300g were given daily subcutaneous injections of 0.6 mg/kg Cd, 5 days per week, for up to 12 weeks. One day each week, 24-h urine samples were collected and analyzed for Kim-1, total protein, Cd and alpha-GST. The results showed that significant levels of Kim-1 began to appear in the urine as early as 5-6 weeks into the treatment protocol and then continued to rise for the remainder of the 12 week treatment period. By contrast, significant levels of Cd and alpha-GST did not appear in the urine until 8 weeks and significant proteinuria was not evident until 10 weeks. These results provide additional evidence that Kim-1 is a useful biomarker of the early stages of Cd-induced proximal tubular injury. Furthermore, they suggest that these early stages do not involve extensive necrosis of proximal tubule epithelial cells. Supported by Grants ES006478 to WCP and DK39773 and KS072381 to JVB.

379 DETECTION OF MULTIPLE GLOBIN MONO-ADDUCTS AND CROSS-LINKS AFTER EXPOSURE OF RAT ERYTHROCYTES TO S-(1, 2-DICHLOROVINYL)-L-CYSTEINE SULFOXIDE BOTH *IN VITRO* AND *IN VIVO*.

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S-(1,2-Dichlorovinyl)-L-cysteine sulfoxide (DCVCS), produced by an FMO3-mediated oxidation of the trichloroethylene (TCE) metabolite S-(1,2-dichlorovinyl)-L-cysteine (DCVC), is a more potent nephrotoxicant than DCVC. DCVCS incubations with N-acetyl-L-cysteine at pH 7.4, 37°C resulted in formation of three mono-adducts and one di-adduct [Chem. Res. Toxicol. (2007) (Epub)]. Because these results suggest that DCVCS could form mono-adducts and cross-links with proteins, globin from Sprague-Dawley rat erythrocytes incubated for 2 h with 0.9-450 μM DCVCS at pH 7.4, 37°C and from rats dosed with 230 μmol/kg DCVCS was subjected to 1D SDS-PAGE and a variety of mass spectrometric analyses to investigate formation of DCVCS-hemoglobin (Hb) adducts. ESI/MS of intact globin chains revealed adduction of 1 and 2 DCVCS moieties on the β1 chain and 3 DCVCS moieties on the α2 chain at the lowest concentrations of DCVCS. Globin dimers were detectable by 1D SDS-PAGE and by ESI/MS at all DCVCS concentrations in vitro and in vivo. LC/MS and MALDI/FTICR of trypsin digested peptides revealed formation of DCVCS mono-adducts preferentially with Cys13 of α chains. LC/MS and MALDI-TOF/TOF also revealed the presence of several peptide cross-links with DCVCS acting as a cross-linker in vivo and in vitro. Although data suggested that Cys125 is the preferential reactive site for cross-link formation between Hb chains in vitro, the in vivo results indicate that other cysteine sites may also be alkylated. Analysis of high accuracy MS data also led to detection of cross-links containing DCVCS-GSH and DCVCS-Cys providing evidence for reactivity of DCVCS with Hb, GSH, Cys, and potentially other biological nucleophiles. Detection of the Hb mono-adducts and cross-links at low DCVCS concentrations may help develop biomarker assays for DCVCS formation in vivo. Furthermore, these results may help elucidate the mechanism behind TCE-induced nephrotoxicity and carcinogenicity. (Supported by NIH Grant DK44295 and NIEHS T32-ES-007015.)

380 PSTC VXDS#2: NOVEL URINARY BIOMARKERS THAT DETECT KIDNEY TUBULAR HISTOPATHOLOGIC ALTERATIONS OUTPERFORM OR ADD VALUE TO BUN AND SERUM CREATININE.

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Nineteen exploratory studies were performed in rat, most of which used nephrotoxicants. Samples were generated from these studies at low, medium, and high dose for up to two weeks in length. Serum samples were tested for BUN and serum creatinine (Cr) levels, while urine samples were evaluated with novel and established biomarkers of renal toxicity, including Kidney Injury Molecule-1 (Kim-1), Trefoil Factor-3 (TFF3), and Albumin. Urinary biomarker concentrations in collected samples were determined by ELISA, Immunoturbidimetric, or Luminex platforms and normalized to urinary creatinine levels and expressed as fold-change compared to study and time-matched control groups. Performance of these five biomarkers was compared to kidney histopathological assessment, which included comparison to a range of histomorphologic changes that included tubular necrosis and degeneration, tubular regeneration and basophilia, and/or tubular dilatation. Histomorphologic composites were performed to refine the correlation between pathology and biomarker values. Pathology was aligned to the Predictive Safety Testing Consortium Lexicon, which allowed data to be compared across companies. Plots of individual animal data showed a high correlation between biomarker levels and the severity of histopathologic change observed. Our kidney biomarker panel detects a majority of histopathologic alterations observed in our study set. For

generalized necrosis and degeneration, Albumin values showed remarkable separation from BUN and serum Cr in a set of nineteen studies in ROC Analysis. Collectively, urinary Kim-1 and Albumin biomarkers outperform BUN and serum Cr in ROC analysis and show greater sensitivity and dynamic range for a variety of nephrotoxicant induced histomorphologic changes. In logistic regression analyses, TFF3 measurements add information to BUN and serum Cr values.

381 EVALUATION OF RAT GST-ALPHA AND OSTEOPONTIN AS MARKERS OF PROXIMAL TUBULAR INJURY.

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Excretion of two proteins, glutathione-S-transferase-alpha (GST-alpha) and osteopontin as biomarkers of proximal tubular injury was examined using rat specific enzyme immunoassays. Male Sprague-Dawley rats were treated with gentamicin (20, 80 and 240 mpk), cyclosporine A (6, 30 and 60 mpk) and Compound A (75, 150 and 225 mpk) for approximately 15 days and with cisplatin (0.5, 3.5 and 7 mpk) for approximately 8 days to induce proximal tubular injury. Urine collections were performed overnight prior to necropsy on Day 3, 8 and 14 from animals treated with either gentamicin, cyclosporine A or Compound A and on Days 3 and 8 from animals treated with cisplatin. For gentamicin, Compound A and cisplatin study, levels of GST-alpha excreted were the highest on Day 3 and generally correlated with the occurrence of tubular necrosis. In the presence of tubular regeneration which generally occurred on Day 8 and 14, urinary GST-alpha levels returned to baseline. Levels of osteopontin in the urine were also increased in the dose groups with histomorphologic changes on Day 3. While osteopontin levels did gradually decrease with continued dosing when tubular regeneration and/or dilatation became prominent, osteopontin levels were detectible and remained elevated at later time points. Tubular basophilia, tubular epithelial vacuolation and outer stripe mineralization were not associated with Osteopontin and GST-alpha elevations on Days 8 and 14 in cyclosporine A-treated animals. These results indicate that GST-alpha may be a good early biomarker of necrosis while osteopontin could serve as a marker of tubular injury when necrosis, regeneration and/or dilatation are observed in the proximal tubules.

382 BIOMARKERS OF FURAN EXPOSURE BY METABOLITE PROFILING OF RAT URINE WITH LC-TANDEM MASS SPECTROMETRY AND PRINCIPAL COMPONENT ANALYSIS.

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Furan has been found in a number of heated food items and is carcinogenic in the liver of rats and mice. Estimates of human exposure on the basis of concentrations measured in food are not reliable because of the volatility of furan. A biomarker approach is therefore indicated. As a first step, we searched for metabolites excreted in the urine of male Fischer 344 rats treated with 40 mg/kg body wt furan by oral gavage. A control group received the vehicle oil only. Urine was collected over two 24-hour periods both before and after treatment and analyzed by a column-switching LC-MS/MS method. Data were acquired by a full scan survey scan in combination with information-dependent acquisition of fragmentation spectra by the use of a linear ion trap. Areas of 449 peaks were extracted from each chromatogram and used for multivariate data analysis. Principal component analysis (PCA) separated the samples of treated rats from the controls in the first post-treatment sampling period. Fourteen peaks were identified in the loadings plot as potential biomarkers and were reanalyzed for quantification. Eight of them showed a highly significant increase upon treatment. Based on the MS/MS information, four of those compounds could be attributed to putative furan metabolites. By means of appropriate in vitro incubations it was possible to show that structures were compatible with glutathione conjugation products and adduct formation with lysine. A neutral loss of 171 was attributed to N-acetyl-L-lysine adducts. Two additional variables showed the characteristic neutral loss for mercapturates. The data show that a metabolomics approach on urinary excretion products allowed identification of putative biomarkers of furan exposure. Further investigations on dose response and analysis in human urine are worth considering.

383 DIFFERENTIAL EXPRESSION OF LIVER GENES IN MICE EXPOSED TO ACRYLAMIDE IN DRINKING WATER.

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Acrylamide (AA) is a rodent carcinogen causing tumors at multiple organ sites. It is also known to be toxic to central nervous system and reproductive tissues. The recent discovery of AA generated during the heating of carbohydrate-rich foods has raised public health concerns because AA may be a potential human carcinogen. Recently, we reported a significant increase in liver cII gene mutant frequency in AA exposed mice. In this study, we evaluated AA-induced gene expression changes in mouse livers. Seven weeks-old, male mice were treated with 0 or 500 mg/l AA dissolved in drinking water for 3 weeks and liver tissues were evaluated for changes in the gene expression profiles. The control animals exhibited normal body weight gain through out the course of the study while the AA-treated mice showed a significant reduction in body weight which was apparent from week 2. Also, the mice exposed to AA consumed significantly less water throughout the treatment. We employed the Phalanx Mouse OneArray for gene expression profiles and performed three technical replicate arrays for each RNA sample. When a cutoff value of a 2-fold change and a P-value less than 0.05 was used as minimum requirements, a total of 696 genes were identified as differentially expressed in AA-treated mice. The effects of AA were mainly related to alteration in the expression of genes involving small molecular biochemistry and the metabolism of vitamins and minerals, lipids, and drugs. Also affected by AA treatment were genes for amino acids for the category of molecular and cellular functions. The expression of genes associated with the functions of diseases and disorders such as cancer, endocrines system, reproductive system, metabolic, and neurological systems were also altered by AA. These results provide further insight into the toxicogenomic mechanisms of AA exposure in mice.

384 EVALUATION OF CALPROTECTIN AS A BIOMARKER FOR DRUG-INDUCED VASCULAR INJURY IN THE RAT.

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Calprotectin (S100A8/S100A9 complex) was identified as a potential biomarker for drug-induced vascular injury (DIVI) based on a systems biology analysis of panomics data through a collaboration between Pfizer and Genstruct. A commercially available ELISA designed to measure calprotectin in human stool samples was evaluated for the measurement of calprotectin in rat serum samples, and then applied to serum samples from animals administered PDE4 inhibitors known to cause vascular injury. The ELISA was initially validated by measuring calprotectin in positive and negative control samples from naïve rats and tested for general assay performance. Once the assay was proven acceptable, it was used to measure calprotectin in serum samples from three studies in which PDE4 inhibitors were administered to rats. The results were correlated with the incidence and severity of mesenteric vascular injury, hematology, gene expression and drug exposure data and systemic levels of other potential biomarkers of DIVI. Analysis of the study samples revealed a statistically significant increase in calprotectin levels in the serum of animals dosed with a PDE4 inhibitor compared to those dosed with vehicle alone. This difference in serum calprotectin levels is observed as early as 6 hours after dosing and may precede the formation of DIVI. In these studies, calprotectin levels did not associate to the severity of the DIVI. The serum calprotectin levels correlated with the level of neutrophils in the blood (calprotectin makes up 60% of cytosolic protein in neutrophils), as well as other potential biomarkers of DIVI (fibrinogen and TIMP-1). A limited data set also demonstrated some correlation between serum calprotectin protein levels and the levels of S100A8 and S100A9 mRNA transcripts in mesenteric arteries. Further studies are necessary to determine if calprotectin, either alone or as part of a panel, is a sensitive, specific and predictive systemic biomarker for DIVI.

385 ASSAY OF VE-CADHERIN GENE EXPRESSION TO MEASURE CIRCULATING ENDOTHELIAL CELLS AS A BIOMARKER OF VASCULAR INJURY IN DOGS.

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Background: An Expert Working Group recommended evaluation of Circulating Endothelial Cells (CECs) as a biomarker for drug-induced vascular injury (DIVI). The lack of suitable CEC antibody reagents has hindered development of a fluorescence-activated cell sorting (FACS) assay for dogs, but quantitative reverse transcriptase-PCR (RT-PCR) provides an alternative method for assay. The vascular endothelial (VE)-cadherin gene is a rational candidate for quantifying CECs because it codes for an adhesion molecule specifically expressed on endothelial cells.
Objectives: Our aim was to develop and assess the assay of canine VE-cadherin gene expression in whole blood to evaluate CECs as a biomarker of DIVI in dogs.
Methods: Custom primers were generated for an RT-PCR assay that was optimized using serially diluted RNA extracted from primary canine coronary artery endothelial cells (cCAEC). A canine kidney cell line (MDCK) was used to evaluate assay specificity. After normalizing VE-cadherin expression data to the 18S gene, relative expression was analyzed. The assay was developed further using normal dog blood spiked with primary cCAECs to evaluate matrix effects. Samples from 10 dogs with mammary cancers and 24 dogs with acute DIVI were evaluated in the study.
Results: The canine VE-cadherin gene assay was specific for cCAECs and sensitive enough to measure RNA extracted from a single cell. In dog blood spiked with primary cCAECs, the limit of detection was 100 cells. In 1 of 10 dogs with mammary

tumors, results were positive for blood samples taken at different time points and tested on separate runs. None of the DIVI blood samples had significant increases in VE-cadherin.

Conclusions: This canine VE-cadherin gene expression assay could be used to measure CECs in tumor-bearing dogs and, with further refinement, potentially in dogs with DIVI. Qualification with a broader sample collection is needed.

Keywords: circulating endothelial cells, VE-cadherin, vascular injury, RT-PCR

386 EVALUATION OF CANDIDATE BIOMARKERS OF DRUG-INDUCED VASCULITIS IN RATS.

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Vascular injury and inflammation are common histological observations in non-clinical toxicology studies conducted to support new molecular entities. Identifying the causative factors of drug-induced vascular injury and distinguishing them from the secondary inflammation as well as correlating effects observed in animals to potential human risk are a difficult challenge. The complexity of the issue creates the need for specific biomarkers of drug-induced vasculitis. Potential serum biomarkers were identified through multiplex protein analysis data mining and literature searches. Candidate biomarkers were then evaluated in serum from SD rats treated with compounds known to induce vascular injury and the results were correlated to standard clinical chemistry parameters and histopathological findings. Through the use of Rules-Based Medicine Rodent MAP® multiplex immunoassay analysis as a primary screen, neutrophil gelatinase associated protein (NGAL) was verified as a candidate biomarker. This protein is secreted by activated macrophages and was elevated for up to 24 hours after drug treatment when histopathologic lesions were observed. Thrombospondin 1 (TSP1) was also identified in the literature as a marker associated with vasculitis. There was an apparent inverse relationship between levels of NGAL and TSP1, with the latter being significantly decreased at the end of study. These results are encouraging and provide the impetus for further characterization of these proteins as potential early signals of vascular damage.

387 CHARACTERIZATION AND UTILITY OF TWO NOVEL PEPTIDE MARKERS OF EXOCRINE PANCREATIC TOXICITY.

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Serum amylase and lipase levels are widely used for the detection of exocrine pancreatic toxicity. Yet, levels of these enzymes are not predictive of disease severity and are known to be poor indicators of early pancreatic injury. Using a proteomic screening strategy, we identified two target organ specific markers of pancreatic toxicity (RA1609 and RT2864). To compare the sensitivity of these novel markers to current standard tests for pancreatic damage, we measured serum levels of amylase, lipase, RA1609, and RT2864 in rats treated with four doses (50-200 mg/kg) of the model pancreatic toxin cyanohydroxybutene (CHB). We then asked whether these peptide markers are useful in the detection of pancreatic injury induced by other toxins and whether these markers were predictive of pancreatic insult across species. Levels of RA1609 and RT2864 proved to be more sensitive than amylase or lipase in detecting pancreatic injury, especially at 8 hr post CHB administration. Changes in marker levels also accurately predicted pancreatic injury induced by caerulein in rats. In mice, which are less susceptible than rats to CHB-induced pancreatic injury, levels of RA1609 and RT2864 proved to be sensitive in detecting even very mild pancreatic injury. Interestingly, a comparison of the RA1609 fragment sequence across species indicated that cleavage of albumin from pancreatic proteases produces a similar peptide marker in several species, including humans. To determine if the human albumin fragments could be detected in sera from patients with various pancreatic diseases, we analyzed sera from normal controls and from patients with diabetes, vasculitis, pancreatic cancer, and pancreatitis. Markers corresponding to the rat fragments RA1609 and RT2864 were present in human serum, and markers levels were indicative of and specific to pancreatitis. In conclusion, RA1609 and RT2864 levels are sensitive indicators of exocrine pancreatic damage and may be useful as safety markers for general pancreatic toxicity in multiple species.

388 PLASMA VON WILLEBRAND FACTOR (VWF) AS A POTENTIAL PRECLINICAL DRUG-INDUCED VASCULAR INJURY (DIVI) BIOMARKER.

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Due to the lack of pre-clinical or clinical non-invasive biomarkers, the drug development process is complicated when there are pathological findings of drug-induced vascular endothelial cell (EC) injury in dog or rat pre-clinical safety assessment studies. In humans, elevation of plasma VWF is considered a marker of EC perturbation/injury but this marker has not been thoroughly evaluated in other species. Using different experimental protocols, plasma VWF was evaluated in rats and dogs to determine if plasma VWF was a biomarker of EC perturbation and specifically DIVI. In the rat, multiple venipunctures increased plasma VWF levels greater than 30% in controls. This was thought to be due to platelets containing high VWF levels, because VWF levels did not increase above baseline when using a Culex automated blood sampling system, which collects multiple blood samples without multiple venipunctures. However, plasma VWF did not increase with treatment that induced vascular injury when using the Culex system. In the dog, repeated venipunctures increased plasma VWF levels less than 5%. This was thought to be due to dog platelets containing less VWF than rat platelets (0.09 and 0.78 platelet to plasma ratio, respectively). Plasma VWF was qualified as an EC perturbation biomarker since physiological stimulation of the EC with 5 ug/kg DDAVP increased plasma VWF levels 54% and pathological stimulation with 2 mg/kg LPS resulted in a 138% increase. Plasma VWF was qualified as a DIVI biomarker in dogs using a potassium channel opener that induced lesions with dose dependent severity. Plasma VWF levels increased 30% at 3 hours post dose in dogs with severe lesions and plasma VWF levels were decreased at 24 hours post dose in dogs with mild, moderate and severe lesions. In summary, VWF requires further investigation in the rat, but qualifies as a potential reporter DIVI biomarker in the dog.

389 IDENTIFICATION OF NOVEL SAFETY BIOMARKERS FOLLOWING EXPOSURE TO THE MODEL PANCREATIC TOXIN CYANOHYDROXYBUTENE.

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Despite their routine use as markers of pancreatic toxicity, serum amylase and lipase levels are often not predictive of histopathology. With the goal of discovering novel biomarkers of pancreatic acinar cell injury, biologic specimens were collected from male IGS rats treated with the model pancreatic toxin cyanohydroxybutene (CHB). Serum and tissue samples were collected at 4, 8, and 24 hr after treatment with 150 mg/kg of CHB. Serum and tissue samples were analyzed by proteomic methods, and these results were compared with findings obtained from standard histopathologic evaluation of pancreatic tissue. Treatment with CHB results in an "edematous pancreatitis" characterized by mild to moderate inflammation, depletion of zymogen granules, acinar cell apoptosis, and with high exposure, secondary necroses occurs. Serum tryptic digests were analyzed by surface-enhanced laser desorption-ionization mass spectrometry. Two novel peptide biomarkers that were predictive of pancreatic damage were identified (RA1609 and RT2864). When compared to basal levels, treatment with CHB resulted in a decreased level of RA1609. In contrast, an increase in the level of RT2864 was observed within 8 h of CHB treatment. The changes in RA1609 and RT2864 were detected in media from CHB-treated primary rat acini, demonstrating that these peptides are either of pancreatic cell origin or are produced by proteases released from acinar cells. Sequence analysis of the fragments revealed RA1609 to be identical to residues 348-360 of rat albumin (accession number P02770), and the sequence of RT2864 was homologous to residues 39-65 of rat trypsin III (accession number P08426). These two peptides, and possibly other identified fragments which result from the enzymatic digestion of circulating proteins by pancreatic proteases, may be useful as safety markers for exocrine pancreatic toxicity during drug development or as biomarkers for the diagnosis and/or grading of severity of pancreatic disease.

390 EVALUATION OF SERUM TROPONIN I AND URINARY MYOGLOBIN AS PRECLINICAL BIOMARKERS OF SKELETAL MUSCLE TOXICITY IN RATS.

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Standard preclinical markers of skeletal muscle toxicity in rats lack sensitivity and specificity. In the present studies, the utility of serum troponin I (fast-twitch; sTnI) and urinary myoglobin (uMB) was evaluated in rat models of myotoxicity. Both proteins were quantified by ELISA, and the data were compared to standard clinical chemistry parameters and histopathological assessments. The limits of detection for sTnI and uMB were 5.9 and 1.6 ng/mL, respectively, and both were below detection limits in control rats. In an acute model of cardiac myotoxicity induced by isoprenaline (0.1-0.5 mg/kg/day), sTnI did not increase, demonstrating its specificity. In chronic models of myotoxicity induced by cerivastatin (0.5, 1 mg/kg), triamcinolone (2.5 mg/kg) and streptozotocin (STZ; 50 mg/kg), dose- and time-dependent increases in sTnI were observed, with female rats being more sensitive than males. In general, when sTnI was increased, neither serum creatine kinase nor aldolase was increased to a similar degree. uMB excretion was increased with cerivastatin-induced myotoxicity but was not increased in a STZ-induced myopathy. In

addition, uMB excretion increased with isoprenaline- and cobalt-induced cardiac toxicity. These data suggest that uMB may be a general marker of muscle toxicity, with little selectivity between cardiac and skeletal muscle damage. In contrast, sTnI appears to be a sensitive marker for skeletal muscle toxicity and can distinguish between fast- and slow-twitch muscle fiber damage. sTnI appears to be a good candidate for inclusion as a preclinical biomarker of muscle toxicity in rats.

391 EARLY CYTOKINE CHANGES AS SURROGATE MARKERS OF LOW DOSE IRRADIATION.

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Following radiological or nuclear terrorism or accident, there is a need for biodosimeters that can triage personnel into treatment categories. Since lung damage may occur from either direct or inhaled irradiation, our group has begun to look at cytokine responses to low doses of radiation following both localized (whole lung) or total body irradiation (TBI) and to correlate any changes in expression with both the early and late pulmonary responses. Groups of 10 C57Bl/6 mice, 6-8 weeks of age, received either whole lung or total body external irradiation at doses of 0, 0.5, 1, 2.5, 5 or 10 Gy. Animals were sacrificed at time points between 1 hour and 15 months. Alterations in cytokine mRNA and protein expression were assessed in tissue (lung and liver), serum and lavage (BAL). In lung tissue, robust, dose response changes were seen in CXCR2 mRNA expression at 1 and 6 hours after irradiation, returning to baseline by 24 hours. These were concurrent with increases in protein levels of KC, a neutrophil chemotactic factor and a ligand for CXCR2, and interleukin (IL)-6 measured in the serum. Total cell counts (BAL) indicated that there is a rapid (>48 hours) dose-responsive decline in total cell numbers. In contrast, in the tissue, there was an abrupt increase in infiltrating neutrophils over the 1 to 12 hour period, followed by an equally rapid decline. These results suggest KC (the murine equivalent to IL-8/GRO) and IL-6 may be candidates for biodosimeters. Log transformation of the values for these two cytokines demonstrated a clear threshold for doses at or above 2 Gy. Their role in the progression of tissue response is unclear at present, nonetheless, their early change in expression may act as a surrogate for lung response. Investigations are currently underway to further examine cytokine expression at later time points and correlate these early changes with late tissue effects. Supported by U19 AI-067733 P01, P30 ES-01247

392 BIOMARKER LEVELS IN DOGS AFTER 28-DAY ORAL TREATMENT WITH SR16157.

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SR16157 is a dual-acting breast cancer therapeutic that inhibits estrogen biosynthesis directly by binding to estrone sulfatase (ES), releasing a selective estrogen receptor modulator (SERM), SR16137, that blocks the estrogen receptor. In a GLP toxicity study, female beagle dogs received SR16157 by oral capsule for 28 days at 0.5, 2.5 or 10 mpk (10, 50, 200 mpm2) with 14 days recovery (Day 42). ES activity was determined in peripheral blood mononuclear cells (PBMC) before and during the study, and in liver collected at necropsy. Plasma drug levels and urinary metabolites were also evaluated by LC-MS/MS. All dogs survived to scheduled sacrifice with no test article-related clinical observations or body weight changes.Various reversible changes relating to white blood cells were observed in SR16157-treated dogs including reduced WBC, ALY, PEO, AEO, and AMO. Mean thymus weights for high dose dogs were decreased 30% compared with controls at Day 29 and remained 20% lower at Day 42. There were no drug-related findings after microscopic examination of all tissues. Hepatic ES activity was completely inhibited in all SR16157 groups by Day 29. PBMC ES activity was completely inhibited by Day 6, remained low through Day 29, and increased by Day 42 inversely proportional to dose. Exposure to SR16157 tended to increase with dose level as indicated by Cmax and AUC. The concentration of the metabolite SR16137 was highest in plasma samples that also contained higher concentrations of SR16157. The presence of SR16157 and monodesethyl-SR16157 was seen in urine collected from dogs in the 2.5 and 10 mpk groups. In conclusion, daily oral administration of SR16157 for 28 days in female dogs was associated with no toxicologically significant effects that would limit clinical use. Furthermore, ES inhibition in PBMCs is a very sensitive indicator of the pharmacologic action of SR16157 and is a potential biomarker of efficacy in human patients. Work supported by NCI Contract N01-CM-42203.

393 TEMPORAL PATTERN OF SKELETAL MUSCLE GENE EXPRESSION FOLLOWING PROLONGED ENDURANCE EXERCISE IN ALASKAN SLED DOGS.

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Muscle injury due to either exertion or adverse drug reactions is typically detected by increases in serum levels of CK, ALT and AST. However, the ability to discriminate exertion-induced from drug-induced muscle injury is not currently possible and is a crucial development issue for all metabolic disease agents that affect lipids or will be used in conjunction with statins. Since conventional biomarkers do not distinguish the benign effects of exertion-induced muscle injury from symptoms preceding the most severe form of drug-induced myotoxicity (rhabdomyolysis), sensitive and discriminating biomarkers are needed. Muscle biopsy specimens and serum were collected at five different time points (prerun, 2, 6, 12, and 24 hours post run) from sled dogs that had just completed a 100 mile training run. Traditional enzymatic assays for two muscle biomarkers in plasma (CK and AST) were measured and showed that animals experienced significant muscle injury post-exercise. CK, AST and to a lesser extent ALT all had significant and time dependent elevations following the run. Gene expression profiling of muscle biopsies revealed very consistent changes in signatures, which exhibited a temporal, time dependent pattern of expression. In general, the greatest number, as well as the magnitude, of gene expression changes, as compared to animals sampled prerun was at 2 and 6 hours, with gene expression going back to basal levels at 12 and 24 hours post exercise. These signatures may provide the first step in developing differentiating biomarkers to distinguish between exertion versus drug-induced muscle injury.

394 A PROTEOMIC APPROACH TO IDENTIFY POTENTIAL TARGET PROTEINS OR BIOMARKERS INDUCED BY THE HSP90 INHIBITOR 17-DMAG IN KASUMI-1 LEUKEMIA CELLS.

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Heat shock protein (Hsp) 90 is an important chaperone protein that regulates the folding of key signaling proteins and maintains their function. Many oncogenic proteins, such as Raf-1, v-Src, Bcr-Abl, c-Kit, that are involved in cell proliferation and survival have been identified as targets of Hsp90 inhibitors for cancer therapy. However, an in-depth understanding of molecular targets or biomarkers of Hsp90 inhibitors remains under extensive investigation. In this study, we employed a global proteomics strategy to identify possible protein signatures affected by 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG) in Kasumi-1, an acute myeloid leukemia cell line that is sensitive to Hsp90 inhibitors. After treatment with or without 17-DMAG at 100nM, the protein expression profiles of Kasumi-1 cells were determined using two-dimensional electrophoresis combined with fluorescent staining and proteomic analysis in different narrow pH strips (pH 3.9-5.1 and 5.5-6.7). The average of total number of protein spots detected by image analysis on fluorescence-stained gel images from Kasumi-1 extracts was ~1300 and 1400 with pI from 5.5 to 6.7 and 3.9 to 5.1, respectively. Compared to the vehicle control, at least 48 protein spots were highly differentially expressed in response to the 17-DMAG treatment. With LC/MS/MS, many known Hsp90 client proteins, such as Hsp70 and its isoforms, hspA8 and hspA1B, were positively identified. A number of possible new target proteins that may be involved in cell translation, folding, signaling, and regulatory proteins involved in microtubule dynamics were also discovered, which are currently under confirmational analysis. Our results suggest that this proteomics-based strategy is well-suited for the identification of target proteins and biomarkers in drug discovery. It may provide new insights into the complex molecular pharmacology of Hsp90 inhibition and may allow a better prediction of the potential activities and toxicities of Hsp90 inhibitors.

395 IDENTIFICATION OF RNS/ROS-MODIFIED PROTEINS AS BIOMARKERS FOR CHRONIC INFLAMMATORY STRESS IN MOUSE LUNG.

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We are currently evaluating whether concurrent exposures to smoke and LPS could produce ROS/RNS modified proteins to serve as biomarkers of response to systemic stress resulting from exposure to cigarette smoke (CS). Groups of 6 AKR/J mice were exposed via nose-only inhalation to either HEPA-filtered air, mainstream CS (250 μg/L wet total particulate matter); 0.5 μg/L LPS for 1 hr/d, 2/wk; or to CS followed by 1 hr of 0.5 μg/L LPS for 1 hr/d, 2/wk for 3 consecutive wk. Lung

was taken for proteomic, transcriptomic and histopathology analysis, BAL fluid, was taken for cytokine analysis. Frozen lung from each animal was digested with trypsin and pooled to provide a sample containing all exposure groups. The pooled peptides were fractionated by strong cation exchange to 20 fractions which were analyzed by OrbiTrap MS/MS. X! Tandem was used to identify peptides containing modifications to the tyrosine residue.
We identified low abundance proteins containing 19 unique nitro-, 13 unique bromo-, and 21 unique chloro-modified tyrosines from the MS/MS database derived from pooled and fractionated lung samples. These modified proteins included surfactant protein A, which directly interacts with peroxiredoxin 6 to protect lung cells against oxidative stress; calumenin, which is highly expressed in the lung and has been observed in BAL fluid from patients with pulmonary fibrosis; E3 ubiquitin protein ligase NEDD4, regulatory protein potentially involved in redox response; beta-catenin, which is a signaling protein in Wnt signaling/developmental pathways and potentially lung remodeling; aquaporin-1, a water channel in the lung; several actin proteins; and Heat Shock Protein 1A, a stress-response marker for acute lung injury. Many of these RNS/ROS-modified proteins may serve as potentially useful mode-of-action or exposure-specific biomarkers. Supported by ES16015 to PNNL, a multiprogram national laboratory operated by Battelle for the U.S. Department of Energy under Contract DE-AC05-76RL01830

396 USING PHARMACOKINETIC DATA TO INTERPRET METABOLOMIC CHANGES IN CD-1 MICE TREATED WITH TRIAZOLE FUNGICIDES.

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Triazoles are a class of fungicides widely used in both pharmaceutical and agricultural applications. These compounds elicit a variety of toxic effects including disruption of normal metabolic processes such as steroidogenesis. Metabolomics is used to measure dynamic changes in endogenous metabolite levels to determine the biochemical impacts of exposure to xenobiotics. Understanding exposure pharmacokinetics (i.e., metabolism and distribution), coupled with changes in the endogenous metabolome, will help link parental and metabolically derived chemical interactions with the normal biological processes occurring in vivo. In the first study, male CD-1 mice were orally dosed with triadimefon (TDN; 50 or 115 mg/kg/d), propiconazole (PRO; 75 or 150 mg/kg/d) or myclobutanil (MYC; 75 or 150 mg/kg/d) in 10% glycerol formal for 4 days. Daily urine samples were obtained and tissues collected on days 1, 3, and 5. A second study was conducted to investigate the pharmacokinetic parameters of each triazole, and in this study mice received a single oral bolus dose of each fungicide (TDN, PRO, or MYC) and tissues were excised at 0.25, 0.5, 1, 2, 3, 6, 12 and 24 hrs post-treatment. Samples were extracted and then analyzed via GC/MS to determine chemical concentrations. Absorption and metabolism of the triazoles fungicides occurred rapidly in vivo. In general, both the parent compound and its degradation products were no longer detectable by 24 hrs post-treatment. Metabolomic analyses of urine, serum and liver were similarly conducted via GC/MS following a two-step derivatization process. Because these compounds are known to disrupt steroidogenesis, the above method was also modified to target steroid levels in serum and liver samples. Ultimately, this research will aid in linking both chemical- and metabolomic-biomarkers following triazole fungicide exposure.

397 INVESTIGATING THE EFFECTS OF DIFFERENT HEPATOTOXINS ON SERUM BILE ACID AND PHOSPHOLIPID PATTERNS.

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Hepatotoxin-induced liver injury is characterised by varying degrees of apoptosis or necrosis. Serum bile acids (SBA) are a sensitive marker of liver damage, indicating leakage or impaired hepatic uptake. Galactosamine (galN) causes hepatocyte necrosis, ANIT produces bile duct necrosis and cholestasis, and allyl alcohol (AA) periportal liver necrosis. A UPLC-MS SBA assay was developed to determine whether these different hepatotoxins produced unique SBA patterns.
Serum was sampled 24 hrs after dosing with galN (415 mg/kg), 48 & 168hrs after AA (120mg/kg) or ANIT (125mg/kg). Methanol-extracted serum was analysed using an Acquity UPLC coupled to a Q-ToF Premier. Histopathological and clinical chemistry data were collected. Data were analysed using Markerlynx and MZmine.
Differences in SBA patterns were observed with each hepatotoxin. After galN administration, taurine-conjugated BA levels were increased significantly from controls ($p=0.001$) and were further amplified with liver damage severity. The 7β-hydroxylated taurine-conjugated BAs, Δ22-TUDCA and TβMCA increased 150-fold and 130-fold respectively, and Δ22-TβMCA 70-fold.
ANIT also increased the 7β-hydroxylated taurine-conjugated SBAs: Δ22-TUDCA 150-fold and Δ22-TβMCA 120-fold, but in contrast to galN, the 7α-hydroxylated BA TCA increased 130-fold. Treatment with AA had a lesser effect on SBA, with TβMCA increasing 14-fold. Quantitatively, Δ22-TβMCA was the major BA after galN treatment (30%) and TCA after ANIT (50%) and AA (40%). Serum phospholipid levels were also affected by hepatotoxin treatment
Treatment of rats with galN or ANIT increased dramatically SBA concentrations and decreased phospholipid levels, but importantly the pattern was different for each hepatotoxin. Mechanisms responsible for these differences are under investigation. These findings illustrate the utility of UPLC-MS as a sensitive tool for probing the metabolic consequences of toxicity.

398 THE HSP90 INHIBITOR 17-DMAG SUPPRESSES LPS-INDUCED UP-REGULATION OF MULTIPLE CYTOKINES IN HUMAN PBMC *IN VITRO* AND IL-12 PRODUCTION IN A MOUSE SEPTIC SHOCK MODEL.

D. Zhou, L. Li, J. Chu, J. Ye, X. Zhang, S. Qin, Y. Wada and J. Barsoum. *Synta Pharmaceuticals Corp., Lexington, MA.* Sponsor: E. Kitayama.

Heat shock protein 90 (Hsp90) plays an important role in inflammation and its levels are elevated during cellular stress. Inhibition of Hsp90 has potential therapeutic applications in the treatment of autoimmune and inflammatory diseases. However, the profile of Hsp90 inhibitors in the modulation of multiple inflammatory mediators remains to be illustrated. In this study, we evaluated and compared the inhibitory effects of dexamethasone (DEX) and an Hsp90 inhibitor, 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG), in lipopolysaccaride (LPS)-induced cytokine release in human peripheral blood mononuclear cells (PBMC) in vitro and IL-12 production in a mouse septic shock model. Human PBMC were isolated from healthy donors. After priming with 800U/ml h-IFNγ, the cells were co-cultured with 1μg/mL LPS with vehicle, DEX or 17-DMAG at 1, 10, and 100 nM for 24 hours. The concentrations of eight cytokines were determined. Cell viability was verified using a cytoxicity assay. The results showed that 17-DMAG suppressed LPS-induced cytokine production with IC50 values of 8nM on IL-1β, 16nM on G-CSF, 20nM on IL-12, 24nM on IL-10, 64nM on IL-6, and over 100nM on IL-8, TNFα, and GM-CSF. In contrast, DEX demonstrated narrower inhibitory effects which were limited to IL-12 (IC50 of 3nM) and IL-10 (27nM). In the mouse septic shock model, Balb/C mice were treated with17-DMAG at 0.5-1.25 mg/kg, i.v. or DEX at 0.1- 0.5mg/kg, p.o.1 hour prior to 10μg/mL LPS (i.v.) challenge. Serum IL-12 concentrations were determined at 3-hr post-challenge. We found that IL-12 production was inhibited with an EC50 of 1 and 0.23 mg/kg in the mice treated with 17-DMAG or DEX, respectively. The data suggest that compared with DEX, 17-DMAG is a broad inflammatory cytokine suppressor and potentially can be used in anti-inflammatory applications involving activation of a wide spectrum of inflammatory cytokines. However, this effect on multiple cytokines could be associated with a risk of targeted adverse effects.

399 PSTC VXDS #3: URINARY BIOMARKER TO MONITOR DRUG- INDUCED GLOMERULAR TOXICITY IN RATS.

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The C-Path PSTC Nephrotoxicity Working Group has worked with the FDA and EMEA under a joint VXDS rolling qualification procedure to submit their research data to justify the qualification of 7 urinary biomarkers to monitor drug-induced kidney injury for specific regulatory use contexts. A number of compounds have been dosed to rodents, with concomitant generation of traditional histopathology, urinalysis and clinical chemistry data and additional novel biomarker data. Compounds included nephrotoxic as well as non-nephrotoxic compounds appropriate for testing sensitivity and specificity. Some nephrotoxic agents resulted in histomorphologic alterations limited to the tubules while others also involved glomerular injuries. ROC analyses (Receiver Operating Characteristic) were performed to compare the performance of each newly qualified biomarker against the current accepted standards of BUN and serum creatinine with careful attention and segregation to regional pathologic involvement. Three biomarkers, urinary Cystatin-C, urinary Beta-2-microglobulin and urinary Total Protein outperform BUN or serum Creatinine and are qualified for detecting early impairment of tubular re-absorption due to glomerular alterations/damage in rat studies.

Glomerular alterations/damage are defined in the standard lexicon as lesions with grade 1 or higher of the type "mesangial cell proliferation", "mesangial cell enlargement", "glomerular vacuolization" or "interstitial Bowman's capsule fibrosis". Further data analyses show that when urinary Cystatin C, urinary B2-Microglobulin, or urinary Total Protein are used together with BUN and serum Creatinine there is statistically significant improvement in detecting glomerular alterations/damage in rat studies compared to BUN and serum Creatinine alone. Considering these results together with noted striking consistencies from published clinical studies, these biomarkers are concluded to be appropriately qualified as tools for bridging findings from pre-clinical studies to the monitoring of kidney safety in human trials.

400 COMPARISON OF COPROPORPHYRINOGEN OXIDASE (CPOX) AND ITS GENETIC VARIANT (CPOX4) IN HUMAN LIVER.

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CPOX catalyzes the 2-step decarboxylation of coproporphyringen-III to protoporphyrin-ogen-IX in the heme biosynthetic pathway. Previously, we identified a polymorphism (N272H) in exon 4 of the human CPOX gene (CPOX4) and showed that the catalytic activity of cloned, expressed and purified CPOX4 was significantly lower (50% less) than that of the wild type in vitro. Here, we compared mRNA and protein levels as well as catalytic activities of CPOX and CPOX4 in human livers to compare of these enzymes in vivo. Sixty five (65) human livers from the University of Washington School of Pharmacy Human Tissue Bank were genotyped for CPOX4. Two were found to be CPOX4 mutant (-/-), 17 heterozygous (+/-), and 46 wildtype (+/+). Gene expression assays measuring total mRNA levels in all 65 livers did not demonstrate significant differences in gene expression among (-/-), (+/-) or (+/+). Western analysis of mitochondrial protein from (+/-) or (+/+) livers showed comparable CPOX protein levels, whereas CPOX4 protein in the 2 mutant livers ranged from 50% to 95% that found in (+/-) or (+/+). In contrast, the catalytic activities of CPOX4 in both (-/-) livers were much lower (~50%) than the mean activities of either wildtype or heterozygous. Additionally, enzymes from (-/-), (+/-) and (+/+) livers were comparably inhibited by Hg^{2+} (10 μM). These results are consistent with in vitro data and indicate that in vivo CPOX4 may be associated with impaired heme biosynthesis, which is limited further by Hg exposure. Supported by NIH ES04696 and ES07033.

401 POLYMORPHISMS IN THYMIDYLATE SYNTHASE GENE ARE ASSOCIATED WITH INCREASED RISK FOR NON-SMALL CELL LUNG CANCER AND THERAPEUTIC OUTCOME IN A PORTUGUESE POPULATION.

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Worldwide, approximately 1.3 billion individuals are currently smokers, and smoking related-diseases are the second major cause of death. Currently, lung cancer is the most common type of cancer in Europe. Until now, cytotoxic chemotherapy has had a limited impact on survival in metastatic non-small lung cancer (NSCLC). Thymidylate Synthase (TS) plays a key role in DNA synthesis and repair. Although several polymorphisms were described for TS gene, only a few functional related polymorphisms were described. Among those, three are the most relevant: in the 5'UTR, a double (2R) and triple (3R) 28bp repeat element (VNTR's) and SNP (G to C substitution) of the 3R allele; in 3'UTR a 6bp variation (ins/del). To evaluate different genetic susceptibility to NSCLC, the polymorphisms were analyzed for a group of control healthy volunteers (n=411) and for smokers NSCLC patients (n=166), by molecular biology techniques. The separate analysis of polymorphisms showed that individuals carrying the 3R allele have a 1.63 higher susceptibility for developing NSCLC (p=0.023) than homozygous 2R. No association was found for the other polymorphisms. However, the combined polymorphisms analysis showed that individuals homozygous 3R carrying ins allele have 3.91 higher risk to NSCLC (p=0.025) if they have 3C allele when compared to homozygous 3G. The pharmacogenetics analysis performed in patients treated with conventional chemotherapy showed no differences in Kaplan-Meier curves related with VNTR's polymorphism. Nonetheless, the SNP G→C and ins/del polymorphism showed differences in median survival time. Patients with 3C allele have higher median survival time (p=0.001) when compared with 3G/3G. Patients with ins allele have higher median survival time (p=0.008) than those homozygous del. Thus, it was demonstrated that TS polymorphisms are associated with an increased risk to develop NSCLC in smoking patients and to the variability of response to conventional chemotherapy.

402 GSTM1 AND GSTT1 POLYMORPHISMS, RESPONSE TO CHEMOTHERAPY AND SURVIVAL IN LUNG CANCER PATIENTS.

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The chemoresistance of lung cancer cells especially against platinum compounds is influenced by several factors including glutathione S-transferases (GST)s. Several polymorphisms in GSTs are associated with changes (or loss) in enzyme activity, sensitivity to chemotherapy and overall patient survival. Accordingly, in this study the association between GSTM1 and GSTT1 gene polymorphisms and response to platinum based chemotherapy and survival of the 139 (127 men and 12 women) patients with advanced-stage (III and IV) non-small cell carcinoma have been investigated. The multiplex PCR protocol was used to determine the GSTM1 and GSTT1 gene polymorphisms. GSTM1 and GSTT1 polymorphisms did not significantly influence the responses to platinum based chemotherapy. No significant associations were noted between the responses of genotypes to chemotherapy and age, sex, smoking status, chemotherapy treatment status, tumor stage or histology. Significant survival was not observed in patients either with GSTM1 (median survival of 17 months for GSTM1-positive genotype and 18 months for GSTM1-null genotype; p= 0.808) or GSTT1 genotype (median survival of 18 months for GSTT1-positive genotype and 16 months for GSTT1-null genotype; p= 0.754). Multivariate analysis also revealed that the adjusted hazard ratio (HR) of death associated with the GSTM1 and GSTT1 genotypes were insignificant as 0.91 (95% confidence interval [95% CI], 0.52-1.59, p= 0.748) and 1.22 (95% CI, 0.65-2.30, p= 0.530), respectively. The protective association was not observed in either genotypes with the response to chemotherapy. These results show that there is no association between GSTM1 or GST1 genotype and response to chemotherapy and survival in the patients with advanced-stage (III and IV) non-small cell lung carcinoma.

403 IMPACT OF GST POLYMORPHISMS ON HEMOGLOBIN ADDUCTS IN SMOKERS AND NON SMOKERS.

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Adducts formed by the reaction of chemicals or their metabolites with hemoglobin provide a means to estimate exposure to chemical carcinogens. The objectives of this study were to develop methods that can be used to measure hemoglobin adducts using liquid chromatography with tandem mass spectrometry with the modified Edman degradation method for adducts with the N-terminal valine residues, and apply these methods to samples from smokers and non-smokers for carcinogenic compounds (or their metabolites) in cigarette smoke. LC-MS methods have been developed for the measurement of cyanoethylvaline (CEVal from acrylonitrile), hydroxyethylvaline (HEVal from ethylene/ethylene oxide), carbamoylethylvaline (AAVal from acrylamide), carbamoylhydroxyethylvaline (GAVal from glycidamide). For simultaneous analysis of adducts, we measured HEVal, CEVal, GAVal and AAVal in hemoglobin from smokers and non-smokers obtained previously in which the impact of GST T1 and GST M1 had been investigated using GC-MS methods for analysis of CEVal and HEVal (Fennell, T. R., MacNeela, J. P., Morris, R. W., Watson, M., Thompson, C. L. and Bell, D. A. 2000. Cancer Epidemiol Biomarkers Prev 9:705-12). CEVal measured by LC-MS agreed well with previous measurements made by GC-MS methods. HEVal and CEVal were elevated in smokers compared with non-smokers. We have previously found CEVal to provide a useful marker of cigarette smoke intake. Both AAVal and GAVal were higher in non-smokers than CEVal, and increased with increasing CEVal in smokers. AAVal ranged from 31-130 fmol/mg globin (mean = 58) in non-smokers, presumably due to exposure to acrylamide in the diet. AAVal increased in smokers to 69-254 fmol/mg globin (means of 124 and 157 fmol/mg globin in 1- and 2-pack/day smokers). The GST T1 and GST M1 genotype did not appear to influence the level of either AAVal or GAVal, suggesting that these enzymes do not play a significant role in the metabolism of AM or GA.

404 ASSOCIATION OF THE OGG1 SER326CYS POLYMORPHISM WITH LUNG CANCER RISK IN A TURKISH POPULATION.

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Lung cancer is the most common cancer with a high mortality rate. The dominant role of tobacco smoke as a causative factor in lung cancer has been well established. Environmental and genetic predisposition can also play an important role in devel-

opment of lung cancer. Cigarette smoke can cause DNA damage through formation of Reactive Oxygen Species (ROS). 8-hydroxyguanine (8-OHG) is one of the major oxidative lesions and repaired by 8-oxoguanine DNA glycosylase (OGG1) which plays an important role in Base Excision Repair (BER) system. 8-OHG is highly mutagenic and, if not excised /repaired on DNA replication, can cause GC to TA transversion mutations. Several studies have been published on the association between lung cancer and OGG1 Ser326Cys polymorphism with conflicting results. Thus, in this study, it was aimed to investigate the relation between OGG1 Ser326Cys polymorphism and the risk of lung cancer and to measure the levels of urinary 8-OHdG as a biomarker of oxidative DNA damage. One hundred sixty-five subjects with lung cancer and 250 healthy control subjects were genotyped by Restriction Fragment Length Polymorphism-Polymerase Chain Reaction (RFLP-PCR) for OGG1 Ser326Cys polymorphism. No association between OGG1 Ser326Cys polymorphism and the risk of lung cancer was found. It was also measured urinary 8-OHdG levels by ELISA immunoassay in patients who did no received any chemotherapy and radiotherapy and healthy control subjects. There was no significant difference between patients and control subjects in urinary 8-OHdG levels.

405 GENE-ENVIRONMENT INTERACTION BETWEEN HOGG1 SER326CYS POLYMORPHISM AND SMOKING ON LUNG CANCER SUSCEPTIBILITY.

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The DNA repair enzyme human oxoguanine glycosylase 1(hOGG1) is responsible for repairing the oxidative DNA base damage 8-hydroxyguanine and recent studies have indicated that the Ser326Cys hOGG1 polymorphism may be associated with an increased risk of lung cancer. However, no interaction effect on lung cancer risk have ever been evaluated between the hOGG1 gene polymorphism and smoking. In the present study we investigated interaction between the hOGG1 Cys/Cys genotype and smoking on lung cancer susceptibility in 1568 Japanese lung cancer patients in a case-only study design. The patients were asked about smoking by means of a self-administered questionnaire, and blood samples were collected to determine their hOGG1 genotype. A statistically significant interaction odds ratio (ORi) between the hOGG1 Cys/Cys genotype and the smoking status of 40 or more pack-years was observed on the risk of squamous cell carcinoma plus small-cell carcinoma (ORi, 4.53, 95 % CI, 1.24-16.58) in women, but not in men (ORi, 0.73, 95 % CI, 0.43-1.26). Although it was not statistically significant, the ORi for all lung cancer was also higher in women than in men. These results suggest that excess risk in female smokers could result from reduced hOGG1 activity due to the hOGG1 Cys/Cys genotype.

406 GENETIC RISK FACTORS IN PROGRESSIVE MASSIVE FIBROSIS IN COAL MINERS.

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Progressive massive fibrosis (PMF) is a chronic interstitial lung disease with a complex etiology. Cytokines, growth factors and cell-surface adhesion molecules play crucial roles in the pathogenesis of pulmonary fibrosis by mediating inflammation, fibroblast proliferation, angiogenesis, and collagen synthesis. A case-control study was conducted to test the hypothesis that single nucleotide polymorphisms (SNPs) within genes involved in inflammatory and fibrotic processes modulate the risk of PMF development. The study population consisted of 648 underground coal miners with demographic information submitted to the National Coal Workers Autopsy Study (NCWAS). SNPs, which influence the regulation of interleukin-1 (IL-1), IL-6, tumor necrosis factor-alpha (TNFα), transforming growth factor beta-1 (TGFβ1), vascular endothelial growth factor (VEGF), intercellular cell adhesion molecule-1 (ICAM-1), and matrix metalloproteinase-2 (MMP-2) genes, were determined using a 5'-nuclease real-time PCR assay from autopsy lung tissue DNA. There were no significant differences in the distribution of any individual SNP or haplotype between the PMF and control groups. However, the polygenotype of VEGF +405/ICAM +241/-IL6 -174 (C-A-G) conferred an increased risk for PMF. These data suggest that single variants of cytokine and fibrogenic genes are unlikely to strongly influence susceptibility to PMF, although the role of VEGF, ICAM-1, and IL-6 polymorphisms in the development of PMF may require further investigation.
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407 RELATIONSHIP BETWEEN ARSENIC-SKIN LESIONS AND THE MET287THR POLYMORPHISM IN AS3MT GENE.

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AS3MT is the key enzyme in inorganic arsenic (iAs) metabolism. AS3MT catalyses the formation of methyl-As (MAs) and dimethyl-As (DMAs) metabolites from iAs. The exonic 14458 C>T (Met287Thr) and intronic 30585 C>T polymorphic variants of the AS3MT gene have been associated with altered profiles of iAs metabolism, specifically, with lower DMAs/MAs ratios in urine of individuals exposed to iAs or *in vitro* systems involving cultured cells or recombinant AS3MT variants. Previous studies have linked increased proportions of MAs in urine to a higher risk of adverse health effects. However, the direct association between the two AS3MT polymorphisms and susceptibility to iAs exposure has never been examined. We have conducted a cross-sectional study involving 122 residents of an iAs- endemic area in Mexico to investigate the potential association between the M287T and 30585 polymorphisms and the occurrence of skin lesions, the marker of chronic As toxicity. AS3MT polymorphisms were evaluated by real-time PCR and urinary As species were quantified by hydride generation atomic absorption spectroscopy (HG-AAS), using a cryotrap for trapping and separation of arsines. A marginally significant difference in the M287T genotype frequency was observed between individuals with and without skin lesions (P=0.07). The risk of developing skin lesions tended to be higher in subjects with the variant genotypes (14458 CT/TT) as compared to wild-type homozygote CC genotype: OR=4.04 (95% CI 0.95-17.3). However this difference was only marginally significant: p=0.055. The 30585 polymorphism was not associated with the presence of skin lesions. Our data suggest that the AS3MT-M287T polymorphism may be associated with an increased susceptibility to adverse effects of iAs exposures. However, further studies in larger populations are needed to study this association. This study was supported by Conacyt grant 50097

408 AMINOLEVULINIC ACID DEHYDRATASE (ALAD) POLYMORPHISM IS ASSOCIATED WITH BLOOD LEAD LEVEL AND HYPERTENSION IN THE NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY (NHANES III).

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Background: Environmental lead exposure has been associated with an increased risk of hypertension. Few studies have addressed the role of aminolevulinic acid dehydratase (ALAD) polymorphism to influence the effect of lead exposure on hypertension. Methods: We examined the role of ALAD polymorphism in the relationship between hypertension and lead exposure using the data of the National Health and Nutrition Examination Survey (NHANES III). Results: The prevalence of hypertension was 22.8% (n=1695). Among persons with and without hypertension the mean blood lead was 3.72 and 2.78 ug/dl, respectively. The prevalence of ALAD2 carriers was 14.7% and 13.4% for people with and without hypertension, respectively. The mean blood lead of ALAD2 carriers was 3.77 ug/dl and 2.53 ug/dl, whereas ALAD1 homozygous carriers had a mean blood lead of 3.73 ug/dl and 2.81 ug/dl in people with and without hypertension, respectively. Regression analysis stratified by lead quartile showed that in the highest lead quartile (>3.8 ug/dl) ALAD 2 carriers had a higher prevlence of hypertension than ALAD1 homozygous carriers (p= 0.0208). The adjusted odds ratios (by race, sex, age, education, smoking status, alcohol intake, body mass index, serum creatinine, serum total calcium and glycated hemoglobin) for ALAD2 carriers was 1.83 (95%CI; 1.11-3.03). Conclusions: Our findings suggest that genetic variation of ALAD is associated with the effect of lead on hypertension.

409 GENETIC VARIANTS IN FOUR METAL-REGULATORY GENES IN AUTISM.

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Autism is a common neurodevelopmental disorder with both genetic and environmental components. Genetic susceptibility to mercury (Hg) toxicity has been advanced as an explanation for autism in a subset of children and has become a widely held public belief. In this model, even "safe" Hg levels could be implicated in the

etiology of autism due to genetic susceptibility that alters metabolism or intracellular compartmentalization. We sought to test this model by thoroughly screening the genetic sequences of four key genes involved in Hg transport and clearance, MT1a, DMT1, LAT1 and MTF1, for variation and any association with autism. LAT1 and DMT1 function in Hg transport and MTF1 and MT1a are inducible by Hg exposure. Using a sample pool of 48 unrelated individuals from both the general and autistic populations we employed Single Strand Conformation Polymorphism analysis and sequencing to screen all of the exons, 5` and 3` untranslated regions, 1kb upstream of the transcript start, and the exonic bordering regions for genetic variations. We have identified and characterized a number of variants in MT1a, DMT1, LAT1 and MTF1. Of the fifty-three polymorphisms identified, fifteen are novel and three are nonsynonymous SNPs. Polymorphisms were evaluated for differences in allele frequencies using Fisher's exact test. Preliminary findings failed to show association with autism for any of these variants. Further analysis with the TaqMan genotyping system in a larger dataset of 224 autistic samples and 224 controls is underway, but has also not yielded an association. These results suggest that variations in the ability to process and transport heavy metals may not play a significant role in the etiology of autism. This work was supported by grants T32 ES007028, NIEHS R01 07331 and T32 MH065782.

410 ASSOCIATIONS BETWEEN THE 5-HTTLPR POLYMORPHISM AND MERCURY FOR MOOD AND BEHAVIOR IN HUMANS.

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Potential effects of polymorphisms in the promoter region of the serotonin transporter gene (5-HTTLPR) has been reported to alter mood and behavior which are also known to be affected by exposure to elemental mercury in humans. A behavioral test battery was administered on the day of urine and buccal cell collection for 164 male dentists (DDS) and 101 female dental assistants (DAs) occupationally exposed to mercury for an average of 19 and 10 years, respectively. Respective geometric mean urinary mercury (HgU)levels in DDS and DAs were 2.52 (2.22)ug/l and 1.98 (1.98)ug/l. DDS and DAs were evaluated separately. Regression analyses controlled for age, premorbid intelligence, frequency of alcohol/week, and education. Statistically significant associations with urinary mercury concentrations ($p<.05$) were found for 15 test measures among DDS and 11 measures among DAs. Genetic status was associated with 5 measures in DDS and 12 test measures in DAs. All mood scores were associated with the polymorphism in both genders. Additive effects were observed for the BEES Finger TappingAlternate and Hand Steadiness Factor1 in both groups, for performance on the BEES SwitchingRate and BEES Finger TappingDom//Non-dom in DDS, and for the BEES Digit Span Forward and Backward, BEES Vigilance Hits, BEES Pattern Discrimination Rate and BEES TrackingScore in DAs. The polymorphism was expectantly associated with mood but operated more broadly to moderate emotional responsivity across other behavioral domains. Controlling for emotional demand in performance did unmask previously unaffected domains within sustained attention and perception. The results add to the evidence of genetic determinants of mood and behavior that potentially could increase susceptibility to Hg toxicity in human subjects.

411 MULTIPLE LOCI SURROUNDING THE APOE GENE ARE ASSOCIATED WITH APOE LEVELS IN POSTMORTEM ALZHEIMER'S DISEASE BRAIN.

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The ε4 allele of the apolipoprotein E gene (APOE) is a risk factor for late onset Alzheimer's disease (AD). Other factors, such as expression level of apolipoprotein E protein (apoE), have been postulated to modify the APOE related risk of developing AD. However, there are contrasting reports on whether apoE levels are associated with ε4 genotype or elevated in AD brain. The aim of this investigation was to determine if multiple genetic loci surrounding the APOE gene are associated with apoE expression level in postmortem AD brain. Postmortem brain from 8 AD and 8 control subjects were collected. Protein and RNA were extracted from 4 separate regions of each brain, which include the cerebellum (CB), hippocampus (HP), frontal lobe (FL) and temporal lobe (TL). ApoE protein levels were measured by western blot and apoE mRNA were measured by QRT-PCR. Expression levels of apoE were then analyzed for their association with SNP genotype within and surrounding the APOE gene. ApoE protein and mRNA levels in postmortem AD brain were not associated with APOE ε4 genotype. Conversely, apoE protein levels were associated with two SNPs located 12 kb upstream of the APOE gene within the TOMM40 gene, and ApoE mRNA levels were associated with one APOE promoter SNP. Additionally, AD HP had the lowest apoE protein level among the 4 AD brain regions; and this difference was not observed in the control brain. In summary, besides the APOE promoter, genetic loci out side of the APOE gene may also modulate apoE expression in the brain. Thus, we speculate that an upstream region within the TOMM40 gene may influence apoE expression and that interactions among multiple genetic loci surrounding the APOE gene may contribute to the complex nature of AD pathogenesis in a brain region specific manor.

412 IDENTIFICATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN THE HUMAN AHR FROM SIX ETHNIC POPULATIONS.

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The mode of action for the biological effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related dioxin-like chemicals (DLCs) involves initial binding to and activation of the cytosolic aryl hydrocarbon receptor (AHR). The functions of the various domains of the AHR are generally conserved across mammalian species although numerous differences have been identified at the molecular level that can affect receptor activity and sensitivity to TCDD and DLC's. For example, the ten-fold strain differences in sensitivity to TCDD between C57BL/6 sensitive mice and DBA2 insensitive mice is the result of a single amino acid polymorphism in the ligand-binding domain of the receptor that produces AHRs with high- and low-affinity for TCDD, respectively. The human AHR is also a low affinity receptor and reports indicate that over 100 AHR genes have been sequenced for polymorphism identification. These efforts have identified five single nucleotide polymorphisms (SNPs) in the human AHR coding sequence with no SNPs in the ligand-binding domain. The majority of these human AHR SNPs reside in the trans-activation domain, and one is in the DNA binding basic region. We sequenced 100 additional human AHR genes in an effort to further identify single SNPs within the open reading frames (ORFs) of the *Ahr* locus. The DNA was sequenced from six ethnic populations that included Japanese, Chinese, European/Caucasian, African American, South East Asian and Hispanic. Five exonic SNPs were discovered; each had been described previously in the literature or public databases. Three of the five SNPs lead to amino acid changes in the AHR protein and two of the five SNPs lead to synonymous substitutions. With these new sequences, over 200 human AHR gene sequences have been analyzed for SNPs. The results indicate that the human AHR gene is stable in the population with only five SNPs and does not contain any SNPs in the ligand-binding domain that would result in a high TCDD affinity human AH receptor.

413 N-ACETYLTRANSFERASE 2 GENETIC POLYMORPHISM INCREASES BREAST CANCER RISK AMONG WOMEN SMOKERS IN NEW MEXICO.

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Breast cancer is one of the most common forms of cancer among women in the United States. Incidence of breast cancer varies among women in different ethnic groups within the Southwest. Smoking alone has been shown to increase the risk for various cancers. Cigarette smoke contains heterocyclic and aromatic amines which can undergo metabolic activation to form intermediates that initiate carcinogenesis. N-acetyltransferase 2 (NAT2) catalyzes both O-acetylation and N-acetylation of these compounds. We explored associations between breast cancer incidence rates and NAT2 genetic polymorphisms in a case-control study from the 4-Corner's Women's Health Study New Mexico site. Subjects were classified as never or ever smokers and NAT2 genotypes were translated into rapid, intermediate, slow, or very slow (NAT2*5/*5) acetylator phenotypes. The smoking and breast cancer association remains a controversial issue and in this study smoking adjusted for age, ethnicity, menopausal status and meat doneness did not increase breast cancer risk (OR, 1.056, 95%CI 0.880-1.267, p=0.56). However, when analysis was stratified by smoking (ever vs. never), NAT2 slow acetylator phenotype increased breast cancer risk among smokers. Using rapid/intermediate NAT2 phenotype as the referent

group with additional adjustment for smoking, slow NAT2 acetylator phenotype showed a 23% increased risk for breast cancer (95% CI, 0.995-1.516, p=0.056). Risk increased significantly for smokers who had the 'very slow' acetylator NAT2 phenotype (OR, 1.907; 95% CI, 1.91-3.054, p=0.0072). Results for nonsmokers were not significant (OR, 1.266; 95% CI, 0.884-1.90, p=0.255). This risk was more pronounced among postmenopausal women smokers (OR, 2.335; 95% CI, 1.302-4.185; p=0.004). Our results indicate that smoking increases breast cancer risk in postmenopausal women with a slow to very slow NAT2 phenotype. Studies were partially supported by USPHS grants R01-CA034627, R01-CA78762 and P30-ES014443.

414 INVESTIGATION OF THE MECHANISM OF INCREASED N-ACETYLTRANSFERASE 1 (NAT1) EXPRESSION IN ESTROGEN RECEPTOR POSITIVE BREAST CANCER.

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Studies of the breast tumor transcriptome have identified classes with distinctive gene expression patterns, raising the possibility of specifically targeted therapies. One of the major identified breast tumor classes corresponds to the ERα(+) group defined by clinical immunopathology. NAT1 is one of the most consistently and highly over-expressed genes in ERα(+) tumors, but it is unknown whether NAT1 expression is regulated by estrogen. We studied 16 ERα(+) and 5 ERα(-) matched breast tumor/normal samples and confirmed that NAT1 mRNA, protein and enzyme activity were all coordinately increased in 12 of the ERα(+) breast tumors. Quantitative PCR was used to rule out NAT1 gene amplification. Quantitative RT-PCR using primers specific for the alternate Nata and Natb promoters showed that increased NAT1 mRNA in tumors is due to transcription originating at Natb. Analysis of RNAs from 6 breast tumor/normal tissue pairs with a quantitative RT-PCR assay specific for Natb heterogeneous nuclear RNA was consistent with increased Natb transcription initiation and not decreased mRNA degradation. We investigated the effect of estradiol on NAT1 expression in the ERα (+) breast cancer cell line MCF-7 and found no direct stimulation of NAT1 by estradiol. The increased expression of of ESR1 and NAT1 in breast cancer may thus be due to the effect of a common co-regulator. XBP1 expression is also significantly higher in ERα(+) than ERα(-) breast cancer in our collection indicating that it might stimulate NAT1 transcription. However, activation of XBP1 by DTT in MCF-7 cells did not increase NAT1 or ESR1 expression. GATA-3 expression is also high in ERα(+) breast tumors. We speculate that NAT1, GATA-3, XBP1 and ESR1 gene co-expression in breast cancer is due to the genesis of these tumors from luminal epithelium in the differentiated mammary gland duct. Partially supported by USPHS grants CA034627 and ES014443.

415 FUNCTIONAL EFFECT OF N-ACETYLTRANSFERASE 1 (NAT1*10) POLYMORPHISM IN DNA ADDUCT FORMATION AND MUTAGENESIS FOLLOWING EXPOSURE TO AROMATIC AND HETEROCYCLIC AMINE CARCINOGENS.

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N-acetyltransferase 1 (NAT1) catalyzes the biotransformation of many aromatic and heterocyclic amine carcinogens. NAT1-catalyzed N-acetylation often serves as an inactivation pathway. If arylamine carcinogens are N-hydroxylated by cytochrome p450 (e.g. CYP1A1), then NAT1-catalyzed O-acetylation leads to the formation of electrophilic intermediates that can form DNA adducts and initiate mutagenesis. This study compares the NAT1*4 reference allele to a putative rapid acetylator NAT1*10 allele. NAT1*10 is characterized by two single nucleotide polymorphisms (T1088A and C1095A) in the 3' untranslated region. NAT1*10 has been associated with increased risk of numerous cancers, including breast, prostate, urinary bladder, colorectal, pancreas, and non-Hodgkin's lymphoma. However, the functional effect of the NAT1*10 polymorphism is poorly understood. In the present work, both transient and stable transfection of pcDNA5/FRT expression vector constructs containing NAT1*4 and NAT1*10 allelic fragments (NAT1 open reading frame plus 885 nucleotides of the 3' untranslated region) were transiently transfected into COS-1 cells and stably transfected into DNA repair-deficient Chinese hamster ovary cells. Transient transfections show that all NAT1 constructs substantially increased PABA N-acetylation activity compared to vector only transfection. The DNA repair-deficient Chinese hamster ovary cell lines also were stably transfected with CYP1A1 to test the functional effects of NAT1*10 on mutagenicity and DNA adduct formation following exposure to aromatic and heterocyclic amine carcinogens. Partially supported by USPHS grants CA034627 and ES014443 and a grant from Philip Morris USA.

416 ROLE OF HUMAN CYTOCHROME P4501A1 AND N-ACETYLTRANSFERASE 2 GENETIC POLYMORPHISM ON THE MUTAGENICITY AND DNA DAMAGE OF THE ENVIRONMENTAL CARCINOGENS 2-AMINO-1-METHYL-6-PHENYLIMIDAZO[4, 5-B]PYRIDINE AND 4-AMINOBIPHENYL.

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Heterocyclic amines such as 2-amino-1-methyl-6-phenyl imidazo[4,5-b]pyridine (PhIP) and aromatic amine environmental carcinogens such as 4-aminobiphenyl (ABP) exert their carcinogenic effects following biotransformation by phase I and/or phase II enzymes. Genetic polymorphisms in metabolizing enzymes such as N-acetyltransferase 2 (NAT2) may modify cancer risk following exposure. Nucleotide excision repair-deficient Chinese hamster ovary (CHO) cells were constructed with stable transfection of human cytochrome P4501A1 (CYP1A1) and a single copy of either NAT2*4 (rapid acetylator) or NAT2*5B (slow acetylator) alleles. Cells transfected with NAT2*4 had significantly higher levels of SMZ N-acetyltransferase (p = 0.0001), N-hydroxy-ABP O- acetyltransferase (p = 0.0001) and N-hydroxy-PhIP O-acetyltransferase (p = 0.0150) catalytic activity than cells transfected with NAT2*5B. The CYP1A1, CYP1A1/NAT2*4 and CYP1A1/NAT2*5B CHO cell lines each showed concentration-dependent cytotoxicity and hypoxanthine phosphoribosyl transferase (hprt) mutagenesis following PhIP treatment, whereas they were not observed in untransfected CHO cells following PhIP treatment. dG-C8-PhIP was the primary DNA adduct formed in the transfected CHO cell lines. Levels were dose-dependent in the order: transfected with CYP1A1 < transfected with CYP1A1 & NAT2*5B < transfected with CYP1A1 & NAT2*4 but did not differ significantly (p>0.05) following one-way analysis of variance. Only cells transfected with both CYP1A1 and NAT2*4 showed concentration-dependent cytotoxicity, hprt mutagenesis, and DNA damage following ABP treatment. These results strongly support activation of PhIP and ABP by CYP1A1 and a robust effect of human NAT2 genetic polymorphism on ABP–induced mutagenesis. Partially supported by USPHS grants CA034627 and ES014443, and a grant from Phillip Morris USA.

417 ROLE OF HUMAN N-ACETYLTRANSFERASE 2 ACETYLATION POLYMORPHISM IN MUTAGENESIS AND DNA ADDUCT FORMATION BY THE AROMATIC AMINE CARCINOGENS 2-AMINOFLUORENE AND 4-AMINOBIPHENYL.

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Aromatic amine carcinogens such as 2-aminofluorene (AF) and 4-aminobiphenyl (ABP) require metabolic activation to form electrophilic intermediates that mutate DNA leading to carcinogenesis. Bioactivation includes N-hydroxylation catalyzed by CYP1A2 followed by O-acetylation catalyzed by N-acetyltransferase 2 (NAT2). To better understand the role of NAT2 genetic polymorphism in AF- and ABP-induced mutagenesis and DNA damage, nucleotide excision repair-deficient Chinese hamster ovary (CHO) cells were stably transfected with human CYP1A2 and either NAT2*4 (rapid acetylator) or NAT2*5B (slow acetylator) alleles. AF and ABP both caused significantly (p<0.001) greater mutagenesis measured at the hypoxanthine phosphoribosyl transferase (hprt) locus in the CYP1A2/NAT2*4 acetylator cell line compared to the CYP1A2, CYP1A2/NAT2*5B, and untransfected cell lines. Both AF and ABP induced hprt mutant cDNAs were sequenced and over 85% of the single-base substitutions were at G:C base pairs. dG-C8-AF and dG-C8-ABP adduct levels were identified and quantified by liquid chromatography-mass spectrometry and were both significantly greater in the rapid NAT2*4 acetylator cell line following treatment with AF or ABP as compared to all other cell lines. DNA adduct levels correlated very highly with hprt mutagenesis for both AF (r2 = 0.94) and ABP (r2 = 0.96), and were in the order untransfected < transfected with CYP1A2< transfected with CYP1A2/NAT2*5B transfected with CYP1A2/NAT2*4. These results indicate the relative importance of genetic polymorphism in NAT2-catalyzed O-acetylation for the mutagenicity of aromatic amine carcinogens. Partially supported by USPHS grants CA034627 and ES014443, and a grant from Philip Morris, USA.

418 ANALYSIS OF TRICHLOROETHYLENE METABOLISM IN A GENETICALLY DIVERSE PANEL OF MOUSE INBRED STRAINS.

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Trichloroethylene (TCE) is a widely used industrial chemical, and a common environmental contaminant. TCE is a well-known carcinogen in rodents and a probable carcinogen in humans. Although the metabolism of TCE is qualitatively similar in mice and man, the toxicity outcomes are thought to differ. Mouse inbred strains afford a unique opportunity to understand both metabolic and genetic basis for differences in responses to TCE. We tested the hypothesis that apparent individual- and organ-specific toxic effects of TCE are genetically controlled and that the mechanisms of toxicity and susceptibility can be successfully elucidated using a panel of mouse inbred strains. TCE (1000 mg/kg) or corn oil vehicle were administered by gavage to 6-8 wk old male mice of eighteen inbred strains. Serum, liver and kidney were collected 2, 8, and 24hr post dosing. Serum samples were analyzed for markers of hepatocellular injury and the profiles of TCE metabolites. Both oxidative metabolites and mercapturic acid pathway conjugates of TCE were measured by liquid chromatography coupled with tandem mass spectrometry. In addition, whole metabolome in the liver, kidney and serum was evaluated by nuclear magnetic response. We observed considerable differences of the levels of the liver injury biomarkers and metabolic differences after exposure to TCE among the strains. These studies provide better understanding of the dose-response and mechanisms of TCE-induced toxicity anchored on metabolism and genotype-phenotype correlations that may define susceptibility or resistance.

419 THE ROLE OF PARAOXONASE (PON1) IN MODULATING TOXICITY OF OP MIXTURES.

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Most environmental and occupational exposures involve complex mixtures. The toxicities associated with such exposures are modified by differences in activity of detoxication enzymes. To address the role of paraoxonase (PON1) in modulating toxicity associated with sequential exposures to organophosphorus (OP) compounds, we used transgenic mice that express either the human PON1-R192 or PON1-Q192 alloform in place of mouse PON1. Chlorpyrifos oxon (CPO), diazoxon (DZO), and paraoxon (PO) are potent inhibitors of carboxylesterase (CaE). We hypothesized that prior exposure to these OPs would increase sensitivity to malaoxon (MO), a CaE substrate, and that the degree of effect would vary among PON1 genotypes if the OP was a PON1 substrate. CPO and DZO, but not MO, are detoxified by PON1. For CPO hydrolysis, PON1-R192 has a higher catalytic efficiency than PON1-Q192. For DZO hydrolysis, the two alloforms have equal catalytic efficiencies. For PO hydrolysis, the catalytic efficiency of PON1 is too low to be physiologically relevant. When wild-type mice were exposed dermally to CPO, DZO, or PO followed 4-h later by different doses of MO, MO toxicity was increased compared to mice receiving MO alone. This potentiation was due presumably to CaE inhibition. Potentiation of MO toxicity by CPO and DZO was greater in PON1 knockout mice, which have greatly reduced capacity to detoxify CPO or DZO. Potentiation by CPO was more pronounced in PON1-Q192 mice than in PON1-R192 mice due to the decreased ability of PON1-Q192 to detoxify CPO. Potentiation by DZO was similar in PON1-Q192 and PON1-R192 mice, which are equally effective at hydrolyzing DZO. Potentiation by PO was equivalent among all four genotypes. These results indicate that PON1 status can have a major influence on detoxication of OP compounds or metabolism of drugs by CaE. Supported by NIEHS ES04696, ES09883, ES09601/EPA: RD-83170901.

420 SUBCELLULAR LOCALIZATION OF SOLUBLE EPOXIDE HYDROLASE REGULATES ENZYME STABILITY AND ACTIVITY.

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Soluble epoxide hydrolase (sEH) is a phase I enzyme, which has been suggested to play a role in inflammation, blood pressure regulation and cardiac hypertrophy, as well as cell signaling, apoptosis and cholesterol synthesis. Recently, a R287Q sEH polymorphism (found in 14% of the human population) has been implicated in several cardiovascular diseases including increased cholesterol levels in familiar hypercholesterolemia, insulin resistance in Type II diabetes, coronary artery calcification in African-Americans and subclinical coronary atherosclerosis. However, the role of R287Q sEH in these diseases is not clear. We have previously demonstrated that R287Q sEH is substantially targeted into peroxisomes, while wild type sEH is primarily localized in the cytosol. In the present study, we show that the peroxisomal localized R287Q sEH is protected from cytosolic degradation, and thus is more stable than cytosolic localized wild type sEH. tSO assays show that the protective role of peroxisomes to sEH helps R287Q sEH maintain higher enzyme activity, which might contribute to its association with cardiovascular diseases. Our results suggest that the subcellular localization of sEH is important in determining its biological function and clinical significance.

421 EXPLORING GENETIC POLYMORPHISMS IN XENOBIOTIC METABOLIZING ENZYMES THAT MAY AFFECT RESPONSE TO ENVIRONMENTAL TOXICANTS.

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Genetic polymorphisms in xenobiotic metabolizing enzymes can modify biological activity and thus affect the fate, internal dose, and ultimate toxicity of environmental chemicals. Characterization of the influence of single nucleotide polymorphisms (SNPs) on phenotype, together with analyses of SNP frequency across major population groups, can inform variability analysis and better define uncertainty in risk assessment. This project investigated the contribution of such polymorphisms to population variability on the function of several key enzymes involved in xenobiotic metabolism including glutathione s-transferase(s), CYP2E1, and CYP2D6. Within each gene family, the number and type of polymorphic variants, the associated effects on enzyme function, and their frequency across major US population groups were analyzed. Monte Carlo-derived distributions indicate a varying degree of associated inter-individual variability. For example, significant variability is evident for GST activity, primarily because the M1 or T1 null (zero activity) genotype is common across population subgroups. A bimodal distribution of GST enzyme activity is also evident among non-null individuals. Similarly, CYP2D6 polymorphisms result in a 9 to 56-fold difference between the poor metabolizer modal median and total population median in enzyme activity. On the other hand, the effect of CYP2E1 polymorphisms on population phenotype variability is less pronounced. Taken together, these analyses provide support for the use of data on polymorphism-based population distributions of activity for certain polymorphic enzymes in risk assessments involving activation or detoxification of xenobiotics. Ideally, such risk assessments would involve physiologically-based toxicokinetic modeling to assess population variability in internal dose and risk. Disclaimer: The views expressed are those of the authors and not the US EPA.

422 QUANTITATION OF NAT1, NAT2 AND NAT3 mRNA IN THE RAT: INVESTIGATION OF TISSUE, GENDER AND AGE-SPECIFIC EFFECTS ON GENE EXPRESSION.

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The human N-acetyltransferases, NAT1 and NAT2, are important phase II enzymes involved in the biotransformation of xenobiotics, and functional polymorphism of the rat N-acetyltransferase gene Nat2 is often considered a model for functional polymorphism of the human N-acetyltransferases in studies of xenobiotic toxicity and carcinogenicity. In this study, sensitive and specific quantitative assays for the Nat1, Nat2 and Nat3 mRNAs that encode the 3 different rat arylamine N-acetyltransferase isoforms were developed and calibrated. Measurements with RNA isolated from 14 anatomically distinct rat tissues showed that both Nat1 and Nat2 have highest expression in colon. The mRNA levels of Nat1 were higher than Nat2 in liver, lung and spleen, but similar in the other tested tissues. Expression of Nat3 mRNA was at least two to three orders of magnitude lower than expression of either Nat1 or Nat2 in all tissues tested. Comparison of Nat1 and Nat2 mRNA expression in colon, liver and lung of male and females rats detected no substantial gender-specific differences. Measurements of Nat1 and Nat 2 mRNA in liver from rats ranging in age from near term to 1 year showed no change in Nat2 levels, but a substantial increase, greater than 4-fold, in Nat1, with most of this increase occurring from 0 to 4 weeks of age. These quantitative evaluations of rat Nat gene expression confirmed that Nat1 and Nat2 genes are the primary determinants of arylamine acetylation activity in the major rat tissues and permit a more precise comparison with the tissue-, gender-, and developmental-specific expression patterns of the cognate N-acetyltransferase genes of humans and other species. Partially supported by USPHS grants CA034627, ES011564 and ES014443.

423 SIZE-DEPENDENT TOXICITY AND UPTAKE OF FLUORESCENT SILICA NANOPARTICLES INTO CELLS.

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Nanoparticle (NP)-based luminescent probes are being investigated for a variety of biological and environmental imaging and detection applications. However, many of the proposed core NPs agglomerate in solution and others, such as quantum dots, are toxic to cells. Previous studies from our lab have shown that fluorescently doped silica NPs were non-toxic to cells at sizes above 50nm. In this study, fluorescein isothiocyanate (FITC)-doped silica nanoparticles (FSNPs) were synthesized via the microemulsion technique and examined for cytotoxicity and uptake. The resulting FSNPs produced a stable fluorescence emission without photobleaching, which is increased in intensity to single organic fluorophores. Three sizes of FSNPs (15, 45, and 73nm) were characterized for size and morphology using TEM. Furthermore, the size in solution was evaluated using dynamic light scattering (DLS). The TEM analysis showed that the size ranges were comparable to the expected manufactured sizes, while the DLS data showed that these nanoparticles do not agglomerate in solution, making them an ideal candidate for a nanoprobe. Furthermore, cell viability was assessed with the MTT assay and demonstrated size- and dose- dependent decreases in viability at high concentrations (>10 μg/mL). However, at low doses (< 10 μg/ml) cell viability was not altered so cellular uptake was analyzed using confocal microscopy. The confocal analysis showed that the NPs were taken up by the cells and resided in the cytoplasm where they could interact with different organelles. The results of this study suggest that FSNPs are promising for studying the mechanisms of cellular uptake and localization, as well as for replacement of traditional organic fluorophores in biodetection applications.

424 ANALYSIS OF SERUM PROTEIN AND NANOMATERIAL INTERACTIONS AND THE EFFECT ON *IN VITRO* TOXICOLOGY STUDIES.

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This investigation was designed to assess whether the presence of serum in cell culture media influenced cell viability between samples during in vitro toxicology studies. Aluminum (Al 50, 80, 120nm), aluminum oxide (Al_2O_3 30, 40nm), hydrocarbon-coated silver (HC-Ag 15, 25, 80nm), polysaccharide-coated silver (PS-Ag 10, 25-30, 80nm), copper (Cu 40, 60, 80nm) and titanium dioxide (varying crystalline structures and sizes). Characterization of nanomaterials was conducted using transmission electron microscopy (TEM) and dynamic light scattering (DLS), for primary particle size/morphology and agglomerate size in solution, respectively. Cell viability (MTS) and cell morphology (CytoViva URI system) studies were performed in conjunction with DLS experiments to evaluate toxicological effects from observed agglomeration changes in the presence or absence of serum in cell culture media. Material-specific surface property measurements were made using X-ray photoelectron spectroscopy (XPS). In summary, our results demonstrate that many metal and metal oxide nanomaterials agglomerate in solution and that depending upon the solution, particle agglomeration is either agitated or mitigated, especially with the Al_2O_3, Al, and smaller Ag nanoparticles. Corresponding toxicity data revealed that the addition of serum to media can have a significant effect on particle toxicity possibly due to changes in agglomeration or surface chemistry. This was seen specifically with both types of Ag particles, with an increase in cell viability from ~30% to ~95% at 25 μg/mL when serum was present in media. Al_2O_3 and Al nanoparticles with serum present showed only moderate increases over samples without serum. The CytoViva images verified the findings with the Ag nanoparticles, showing nearly normal morphology when particles were dosed with serum in media. The Al_2O_3 particles exhibited less tendency to adhere to the cell membrane when serum was present.

425 EFFECTS OF TEMPERATURE, TIME, AND SOLVENT ON NANOPARTICLE AGGLOMERATION.

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Since previous studies from our laboratory have shown that the environment nanomaterials are in can alter nanoproperties, it is imperative to thoroughly characterize nanoparticles at each stage in the experimental process. Therefore, prior to nanotoxicity studies, we need to address how different solvents and temperatures can impact nanoparticle behavior. The purpose of this study was to examine the effect of increased temperature and time on nanoparticle agglomeration. The nanoparticles used in this study were: silicon dioxide (SiO_2) 35nm, 51nm, 110nm, and 420nm, copper (Cu) 40nm, 60nm, and 80nm, and silver (Ag) 25nm, 55nm, and 80nm. Initially, TEM analysis was performed to verify the manufacturer's size and determine the size distribution range for the particles. The TEM analysis showed that the primary size distributions for the SiO_2 nanoparticles were similar to the manufacturer's size. For the Cu nanoparticles, the ranges were not similar with Cu 40nm 70.3 + 17.8, Cu 60nm 79.7 + 21.3, and Cu 80nm 110.6 + 26.6. For the Ag nanoparticles the only particle not similar to the manufacturer's range was the Ag 80nm with a size of 122.32 + 60.366. The nanoparticles were dispersed in sterile water or exposure media (EM) (media without serum) and stored at 4°C or 37°C. Using dynamic light scattering (DLS) and Cytoviva ultra resolution imaging system, agglomeration was measured at 0, 8, 14, 22, 32, and 48 h to determine if the magnitude of agglomeration was temperature or time dependent. Our results show that SiO_2 35nm and 51nm agglomerated only in exposure media at 37°C with an average size of 2 μm, while the Cu 80nm agglomerated in sterile water at 4°C and 37°C up to 20 μm, and Ag 25nm agglomerated in EM immediately after dispersion and the particles remained agglomerated at 4°C and 37°C with an average agglomerate size of 1.5 μm. Based on this data, time in solution and temperature appear to impact nanoparticle agglomeration which should be taken into account in nanotoxicity studies.

426 DEVELOPMENTAL TOXICITY OF QUANTUM DOTS IN ZEBRAFISH: INFLUENCE OF SURFACE CHEMISTRY.

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Quantum Dots (QDs) have received national attention due to their prospective applications in the biomedical and life sciences fields. While QDs promise to significantly benefit society, most are heavy metal chalcogenides raising concern about the potential risk to human health, wildlife, and the environment. Little information currently exists on the toxicity of QDs in vertebrates or the influence of different surface chemistries on toxicity. We used embryonic zebrafish to assess the developmental toxicity of waterborne QDs and the physiochemical properties influencing their toxicity. Embryos were exposed to poly-L-lysine wrapped CdSe quantum dots with and without a ZnS shell, as well as CdSe/ZnS core-shell QDs functionalized with polyethylene glycol of two molecular weights (PEG350 and PEG5000) and different terminal groups (PEG5000–OCH3, –COOH and –NH3). Preliminary results indicate the stability of QD suspensions were stable in water under our experimental conditions. We demonstrate that the ZnS shell protects embryos from Cd-like sublethal toxicity, and that the terminal functional group contributes to their overt toxicity, perhaps by influencing accumulation and/or elimination. For most of the QDs, observed sublethal toxicity correlated with increased expression of metallothionein and Cd2+ levels in the larvae, supporting the hypothesis that QD toxicity results in part from exposure to Cd2+. Co-exposure with GSH partially rescued the toxicity of some QDs, suggesting that oxidative stress may also contribute to their toxicity. Our work illustrates utility of the zebrafish embryo as a medium-throughput, low-cost, whole-animal, vertebrate model to screen nanomaterials for developmental toxicity. Results from this assay may alert investigators to possible adverse effects to other vertebrates, including humans. (Supported by NSF and UW-NSEC).

427 ZEBRAFISH AS AN ALTERNATIVE MODEL TO ASSESS BIOMATERIAL NANOTOXICITY.

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The rapid development of nano-sized materials for biomedical diagnoses and therapeutic delivery necessitates a rigorous model to bridge putative *in vitro* and *in vivo* paradigms to evaluate nanotoxicity and predict therapeutic efficacy. Recognized as an attractive model in developmental biology and molecular genetics, the zebrafish is an ideal model organism to identify nanomaterial-induced abnormalities through embryonic exposures. To validate our macromolecular nanotoxic predictive model, colloidal gold was chosen as a representative biomaterial due to its stability and controlled physicochemical versatility. Graded concentrations (25-250 μM) of non-functionalized gold nanoparticles (Au NPs) of various sizes (3-100 nm) were microinjected or waterborne-exposed to zebrafish embryos from 6-120 hours post-fertilization (hpf). Every 24 hours, embryos were scored for severity of morphological defects and evaluated for hatching success rate, oxidative stress, and apoptosis. Preliminary studies show that toxicity is size- and concentration-dependent, with the zebrafish chorion acting as a protective barrier against toxicity from 0 to 72 hpf. Tissue dispersion of Au NPs was determined by electron microscopy and neutron activation methods. To further show the utility of the zebrafish model in

assessing toxicity, embryos were exposed to graded concentrations of two established polymeric carriers: BPEI and ELP. BPEI is a highly cationic synthetic polymer and ELP is an elastin-like polypeptide possessing a unique thermotransitive profile. At concentrations below 25 µM the ELP did not induce toxicity in 120 hpf embryos; however, 100% mortality was observed at 84 hpf with BPEI at a concentration of 0.1 µM. It is significant that these results closely parallel *in vitro* and *in vivo* results in the literature. The data thus far support our hypothesis that a zebrafish model can accurately predict nanomaterial toxicity and accelerate pre-clinical drug development. We are confident that zebrafish could ultimately become the "go-to" organism for medium/high throughput nanotoxicity screens.

428 DIFFERENTIAL TOXICITY OF METALLIC NANOPARTICLES TO AQUATIC SPECIES.

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Increasing utilization of metallic nanomaterials in recent years implies an increasing rate of release to the environment, with potentially serious adverse effects on environmentally important species. We have previously demonstrated that nanoparticulate metals vary widely in their ability to cause acute toxicity to aquatic species. Nanoparticulate copper and silver are toxic to Danio rerio (48-hr LC50 = 0.94 mg/L and 7.1 mg/L, respectively), while other metals tested (nano - aluminum, cobalt, titanium dioxide, and nickel) induced no mortality by 48 hours. The observed toxicity is not mediated by dissolution of particles. Exposure to nanosilver results in significantly higher gill tissue burdens (55 ng/mg) than does exposure to nanocopper (9.75 ng/mg), indicating uptake of nanosilver but not nanocopper by gill tissue. Additionally, nanocopper and nanosilver induce dramatically different transcriptional response in gills following microarray analysis. Together, these data indicate that nanocopper and nanosilver may be inducing toxicity through different mechanisms. Particle-mediated reactive oxygen species (ROS) production has been proposed as a potential mechanism controlling nanoparticle toxicity. To determine if differential ability of nanometals to produce ROS could explain the observed differences in toxicity, we measured ROS production by nanoparticulate forms of copper, silver, cobalt, aluminum, nickel, and titanium dioxide in aqueous suspension. Our results indicate that nano-titanium dioxide produces elevated levels of superoxide radicals while nanocopper produces elevated concentrations of peroxide radicals. Nanosilver does not produce significant levels of either superoxide or peroxide. This data suggests that differences in ROS production may help explain differences in toxicity and supports the hypothesis that nanocopper and nanosilver cause toxicity via different mechanisms.

429 A COMPARISON OF BULK AND NANO-SIZED ALUMINUM OXIDE (AL_2O_3) ECOTOXICITY TO *HYALELLA AZTECA* AND *TUBIFEX TUBIFEX* IN SPIKED SEDIMENT.

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There are many uses of nano-aluminum oxide (Al_2O_3) including uses in coatings and industrial abrasives, polishing of optics and jewelry, and as a drug delivery system. Nano-Al_2O_3 also has military applications such as use in propellants and explosives (nano-energetics). However, there has been little characterization of the potential effects of nano-Al_2O_3 to aquatic organisms. I compared sediment toxicity of bulk Al_2O_3 (diameter = 50-200 µm) with nano-Al_2O_3 (diameter = 11 nm) using sediment toxicity tests. Ten-day sediment bioassays were carried out using the amphipod *Hyalella azteca* and the sludge worm *Tubifex tubifex* using a spiked reference sediment. Treatment levels were 10,000, 25,000, 50,000, and 100,000 mg/kg of bulk and nano-Al_2O_3 plus a control. Endpoints assessed include survival, growth, and bioaccumulation for *H. azteca* and survival and bioaccumulation for *T. tubifex*. No significant mortality was observed for either organism at any treatment level. Significant reductions in *H. azteca* growth were observed at the 50,000 and 100,000 mg/kg treatment levels for nano-Al_2O_3; however, no reductions in growth were observed at any treatment level of bulk-Al_2O_3. Thus nano-$Al_2O_3$was more toxic to the *H. azteca* growth endpoint; however, effects were seen at sediment concentrations higher than those likely to be found in the environment, indicating low environmental risk to this endpoint in environmental exposures.

430 THE EFFECTS OF METAL NANOPARTICLES AND FULLERENES ON OYSTER HEPATOPANCREAS CELLS AND EMBRYOS.

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There are numerous potential environmental risks of engineered nanoparticles that are not yet well-characterized or understood. Nanoparticles may be introduced into aquatic environments during production processes and also as a result of release following their use in electronic and biological applications. Filter-feeding bivalve mollusks such as oysters spend their lives removing particles so they are potentially very valuable as model species for characterizing nanoparticle bioavailability and interactions with basic cellular processes. The purpose of these studies was to characterize the toxicity of various nanoparticle preparations (e.g. quantum dots, fullerenes, and silver nanoparticles) on isolated hepatopancreas cells and embryos of oysters, Crassostrea virginica. The potential for hepatotoxicity was evaluated using a lysosomal destabilization assay, and cell viability assays (using flow cytometry) with isolated hepatopancreas cells. For the embryo assays, newly-fertilized oyster embryos were exposed to the nanoparticles and then the percent normal development after 48 hours was assessed. Generally, the metal nanoparticles were more toxic than the fullerenes with both the hepatopancreas assays and embryo development assays. Fluorescent confocal microscopy and electron microscopy were used to verify the accumulation of the nanoparticles inside hepatopancreas cells and embryos. These kinds of basic studies are essential for addressing the potential impacts of nanoengineered particles on fundamental cellular processes as well as aquatic organisms.

431 DIFFERENTIAL AQUATIC TOXICITY RESPONSES OF GREEN ALGAE TO VARIOUS NANOPARTICLE-TYPES.

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Although there is a paucity of data related to the human health effects of nanomaterials, there is, perhaps an even larger data deficit for the environmental implications of nanomaterials. This study examined the effects of various nanoparticle systems in single-cell sterile aquatic environments. The nanoparticle-types evaluated included nano and corresponding fine metal colloids (silver, Ag), nano and fine metal-oxide nanoparticles (aluminum oxide, Al2O3), and an organic nanoparticle (hydroxlyated fullerene, C60(OH)24). These are representative of the wide distribution of nanoparticle-types within the nanotechnology arena. Using a traditional aquatic/eco-toxicity test model, we assessed the response of the green alga, Pseudokirchneriella subcapitata, to these two fine particle, and three nanoparticle-types, each at 3 concentrations. In sterilized 250 mL Erlenmeyer flasks, inoculated test cultures (cell density = 10,000 cells/mL) were incubated in 50 mL of algal medium (AAP) under continuous light (4200-5810 lux). Aliquots of algae and media inoculated with fine and nanoparticle suspensions were analyzed for cell density using two techniques, i.e., via light (optical) microscopy, as well as by flow cytometric analyses at 24, 48, and 72 hours. When compared to control cultures, results showed that at the lowest dose, 3 mg/L, both metal nanoparticle-types but not the fine silver metal colloids or fine aluminum metal oxide particles decreased algal growth at 72 hours; while the fully hydroxylated C60 nanoparticle had less of an impact on depressing algal growth. Effects were dependent upon nanoparticle concentrations in the algae media. Flow cytometric analyses generally were consistent with optical cell count assessments. These results demonstrate the range of variable responses of green algae to different types of fine and nanoparticle-types and indicate the need for careful assessment of other nanomaterials.

432 FATE OF FULLERENE NANOPARTICLES AND THEIR INTERACTIONS WITH ORGANIC MATTERS IN AQUATIC ENVIRONMENTS.

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The wide applications of fullerene and its derivatives in pharmaceuticals and industries will create a new type of environmental contamination. One of the major concerns is the fate of the nanomaterials in aquatic environments. The pristine fullerenes are hydrophobic, insoluble in water and have strong tendency into hydrophobic organic matters, while fullerenes could aggregate to form nanoparticles (nano-C60) soluble in aquatic environments. Due to the extremely high chemical stability, fullerenes could become persistent environmental contaminants regardless of the original applications in compositions or derivatives. The environmental fate of fullerene nanoparticles, their transport mechanism and interactions with organic matters in the aquatic environments were studied in this research. The absorption kinetics of nano-C60 by the organic matters, the effects of salts, pH and temperature were studied by spiking different concentrations of nano-C60 into organic matter containing solutions under a variety of experimental conditions. The ion-pairing effects on the absorption kinetics were addressed particularly to reveal the possible fates of the nanomaterials in different aquatic environments. A surfactant-dialysis method was used to prepare nano-C60; and the nanoparticle sizes were characterized by scanning electron microscopy and dynamic light scattering. The aqueous samples and organic matters were extracted with toluene using our simplified liquid-liquid extraction method and the solvent extracts were concentrated and analyzed by HPLC with 60% toluene and 40% acetonitrile as mobile phase.

433 AN EZ METRIC FOR EVALUATING NANOMATERIAL-BIOLOGICAL INTERACTIONS.

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Nanotechnology will provide a broad range of beneficial applications in the electronics, healthcare, cosmetics, technology and engineering industries due to the unique physical and chemical properties inherent to nanomaterials. Pertinent to the development of nanotechnologies, and to the safety of novel nanomaterials, is a thorough understanding of nanomaterial-biological interactions. Yet, the principal characteristics that may be predictive of nanomaterial interactions with biological systems have not been elucidated because of the current lack of toxicological data and the enormous diversity of nanomaterials. The embryonic zebrafish model is a dynamic *in vivo* system that offers the power of whole-animal investigations (e.g. intact organism, functional homeostatic feedback mechanisms and intercellular signaling) with the convenience of cell culture (e.g. cost- and time-efficient, minimal infrastructure, small quantities of nanomaterial solutions required) to rapidly evaluate interactions between engineered nanomaterials and integrated living systems. Investigations using this model system can reveal subtle interactions at multiple levels of biological organization (i.e. molecular, cellular, systems, organismal) so we have developed an 'EZ (embryonic zebrafish) metric for nanomaterial toxicity' (EZMNT) that takes into account the types and frequency of sublethal effects in addition to overt mortality. The EZMNT was used to compare morbidity and mortality elicited from exposure to a wide variety of engineered nanomaterials (dendrimers, viral nanoparticles, Au and Ag nanoparticles, fluorescent nanomaterials, ceramic nanoparticles, nanoparticulate metal oxides, and carbon nanomaterials – C60, C70, single-walled carbon nanotubes, functionalized single-walled carbon nanotubes, and multi-walled carbon nanotubes).

434 SCREENING ASSAYS FOR ASSESSING NANOPARTICLE TOXICITY.

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With the large number of various nanoparticles being developed and implemented into numerous and diverse commercial applications it becomes necessary to develop effective means of assessing the potential hazard of the material and the associated risk. We determined whether a cell free or in vitro screening assay can be used to predict acute nanoparticle toxicity seen in vivo. To accomplish this we utilized two cell free and two cellular based assays to evaluate their power in predicting in vivo toxicity. Eight different particle types were used ranging in size from 25 to 200nm. The cell free systems utilized a fluorescent dye based assay and electron spin resonance. The cellular systems also used electron spin resonance as well as a cell line with a luciferase reporter construct. Results obtained from these assays were compared with lung lavage PMN content obtained from in vivo experiments. A common response metric for all assays was release/induction of reactive species normalized to the dose unit. Results indicate that nanoparticles have an intrinsic reactive species inducing capacity which in biological systems can lead to oxidative stress. The particles with the greatest activity in all of the assays were copper nanoparticles, while the least reactive particles varied according to the assay, but were frequently comprised of titanium dioxide, gold or polystyrene. The presence of a biological component in the screening assay provides a more reliable estimate of the toxicity when compared with cell free analysis (R-square 0.88, $p<0.0039$ vs. R-square 0.48, $p<0.2329$). For a meaningful analysis it was important to normalize the observed response in each assay to a unit dose in order to allow comparison of outcomes from multiple assays, each of which utilized a different dose/concentration. We conclude that in vitro assays have the potential to be useful in predicting the acute in vivo pulmonary response following nanoparticle exposure. Supported by NIEHS T32ES07026, ES 01247, AFOSR grant FA9550-04-1-0430, EPA STAR PMCenter R-827354

435 LIVE-CELL ATTENUATED TOTAL REFLECTION - FOURIER TRANSFORM INFRARED SPECTROSCOPY (ATR-FTIR) OF THE CELLULAR RESPONSE ON EXPOSURE TO NANOMATERIALS.

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Live-cell attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR) is a potentially valuable screening tool for evaluating and understanding biological responses of cells on exposure to nanomaterials in real time. We have studied the activation by lipopolysaccharide (LPS) as a model proinflammatory stimulus in macrophage cells (RAW 264.7) grown on the ATR crystal. The proinflammatory state is characterized by upregulation of cyclooxygenase-2 (Cox-2) and inducible nitric oxide synthase (iNOS). This mode of action has been widely implicated in nanomaterial toxicity. The data indicate a marked increase in a peak detected at wave number 1652, characteristic of the protein carbonyl bond. Pretreatment of the cells with indomethacin, a Cox-2 inhibitor, fully suppresses the increase in this peak, suggesting a link between this spectroscopic change and Cox-2 activity. We have explored this link further by over expressing Cox-2 in RAW 264.7. Treatment of Cox-2 expressing cells with arachidonic acid, the substrate for Cox-2, is associated with an increase in the peak occurring at the same wave number 1652, providing additional evidence linking this observable live-cell ATR-FTIR change to Cox-2 activity.
We have also studied settling of 1 micron silica on the cell as well as interaction of 10 nm silica with the cell. We now demonstrate that ATR-FTIR can observe and quantify the settling of 1 micron silica particles with rates that tightly match predictions made using a physical particokinetic model. We have completed an initial evaluation of the spectra using advanced statistical methods, including principal component analysis (PCA). Our results indicate that the spectra associated with endotoxin and 10 nm silica-treated cells can be clearly resolved using PCA. This research was conducted under the Laboratory Directed Research and Development Program at PNNL, a multiprogram national laboratory operated by Battelle for the U.S. Depart¬ment of Energy under Contract DE-AC05-76RL01830.

436 INTEGRATION OF MICROARRAY AND PROTEOMIC DATA REVEALS PATHWAY-SPECIFIC INFLAMMATORY MARKERS OF NANOPARTICLE EXPOSURE TO MOUSE MACROPHAGES.

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The advancement of nanotechnology and nanoscale materials has outpaced our understanding of the potential human health risks of these materials. Unbiased approaches are needed to identify new suites of biomarkers that are predictive of nanoparticle toxicity to guide development and regulation of safe nanomaterials. Our research strategy is to distinguish the cell processes and signaling networks activated by noncytotoxic inflammatory stimuli from those that are indicative of nanoparticle toxicity for the identification of new pathway-based biomarkers for high-throughput screening. Global microarray and proteomic technologies were used to evaluate the early response pathways and downstream extracellular protein biomarkers of nanoparticle exposure, using a macrophage cell line model system. Dose response studies were conducted with nano-sized amorphous silica, crystalline silica, single-walled carbon nanotubes and lipopolysaccharide (LPS) to provide parallel samples for gene expression profiling at 2hr post-exposure, global proteomic analysis of secreted proteins after 24hr, and cytotoxicity assessment at 24hr. We identified >500 genes that are significantly regulated following particle exposure but unaffected by LPS. These genes represent many cell processes, including cell cycle regulation, response to stress, nucleotide metabolism and apoptosis. Global proteomic analyses of secreted proteins confirmed many of the inflammatory markers identified in the microarray data, including divergent response pathways characterized by markers unique to particle exposure (HSPA8, NDRG1) and unique to LPS exposure (CSF3). Other identified protein effectors were associated with macrophage survival (MIF), proteolysis (ITIH2), cell adhesion/migration (LGALS3), complement activation (C3, C4B) and regulation of immune response (FKBP4) only after particle exposure. We are using these data to infer signaling networks that capture the critical biological pathways involved in the cell response from nanoparticle exposure.

437 CORRELATING TOXICITY OF NANOMATERIAL WITH PHYSICAL CHARACTERIZATION USING AN OXIDATIVE STRESS MODEL.

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Nanotechnology is rapidly expanding and some estimates have predicted a $1 trillion market by 2015. Manufactured nanomaterial (NM) are already in use through products such as cosmetics, sunscreens, sporting goods, tires and some future medical applications which includes imaging, diagnosis, and drug delivery. The novel properties of NM raise the possibility that they could interact with cellular tissue and cause damage to biological systems. With this in mind I aim to further develop a system whereby NM toxicity can be classified utilizing the Hierarchical Oxidative Stress Model. Due to the large number of new NM being produced each year it would be impossible from a time and cost perspective to test each on its own. For these reasons it is one of my aims to correlate the biological outcome of the tested NM with its physico-chemical properties with the ultimate goal of understanding basic nano/bio principles which may predict future toxic outcomes.

In recent work we have classified several NM on their ability to generate Reactive Oxygen Species (ROS) in a macrophage cell line using air pollutant particles as a positive control. Interestingly, TiO2, Carbon Black and Fullerol were void of toxicity while the NH2 modified polystyrene NP tested positive in cytotoxicity as compared to the COOH or unmodified versions. The mechanism for this toxic outcome has been correlated to its small size and positive charge. Furthermore, this nano/bio interaction is cell specific when tested in 5 different cell lines and can be related to its mechanisms of cellular uptake. Taken together these data have helped establish a method of toxic detection utilizing the Hierarchical Oxidative Stress model for future NM. I will further use this system to categorize a set of Fullerene derivatives which is a good candidate due to their high volume of production and conflicting literature data about the potential toxicity. As nanotechnology develops, it is essential that the toxicological approach also evolves and stays up to date. This will provide an important safeguard for the continued expansion of the nanotechnology industry.

438 NANOMATERIALS TOXICITY: CLASSIFICATION USING MULTI-CRITERIA DECISION ANALYSIS.

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Potential toxicity of nanomaterials and resulting risks at different stages of product life cycle (e.g., development, production, use and disposal) are widely unknown and may depend on last minute changes in engineering design and functionalization. To guide nanomaterial research and applications, as well as its safe use, we developed a decision support system for classifying nanomaterials into different risk categories. The classification system is based on a comprehensive set of performance metrics that measure both the toxicity of nanomaterials and the expected environmental and human health impacts through product life cycle. The stochastic multi-criteria acceptability analysis (SMAA-TRI) sorting method, a formal decision analysis tool, was used as the foundation for this classification system. It allows incorporating expert estimates with experimental and modeling data on nanomaterial physico-chemical characteristics, associated uncertainties and life-cycle impacts. SMAA-TRI expedites analysis of uncertainty by using Monte Carlo simulation to explore the spaces of feasible values for expert-assigned criteria weights, experimental measurements and modeling results. The results provide decision makers with a probabilistic characterization of nanomaterial properties for risk management purposes. The application of this classification system is illustrated for several nanomaterial classes, including titanium dioxide (TiO2), nC60 (a fullerene), single-walled carbon nanotube (SWNT), single-walled carbon nanotube-by product (SWNT-BP), and Quantum Dot (CdSe Core).

439 ANALYSIS OF SIZE-DEPENDENT CELLULAR RESPONSES TO SILICA NANOPARTICLES FROM INTEGRATED GENOMIC AND PROTEOMIC DATA.

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Identifying the biological pathways activated by nanomaterials using unbiased and comprehensive approaches has become a subject of increasing importance for the safe advancement of nanotechnology. A fundamental question is whether the biological responses to an engineered material changes significantly as the material approaches the nano-scale. To address this we are using integrated global genomic and proteomic analyses to define and compare the signaling networks induced in RAW 264.7 macrophages by different diameters of amorphous silica. We find that the ability of unopsonized silica particles to induce overt cell death in macrophages over 24 hr follows a predictable relationship with the total administered particle surface area across a wide range of particle diameters (7-300 nm). Quantitative analysis by protein microarray ELISA show a similar relationship with total administered surface area for several secreted proteins, including TNF, VEGF and G-CSF in response to particles ranging from 10-300 nm. Dose-response studies comparing transcriptional profiles after acute (2 hr) exposure to 10 nm or 500 nm silica were also conducted using whole genome microarray. Pearson correlation analysis indicates that overall there is a high degree of similarity in the genes altered by both particle sizes when compared using similar surface area dose metrics. Pathway enrichment analysis using the integrated data also demonstrated a high degree of similarity in the major cell processes activated by small (10nm) and large (500 nm) particles. However, for a significant fraction of the genes that are induced by both sizes of particles, dose-response patterns based on particle mass dose were identified which do not follow a clear relationship with total particle surface area. These results suggest that for different subsets of biological responses, there are size-dependent modes of action which may reflect differential mechanisms of particle uptake or localization of action.

440 ENHANCED CYTO- AND GENOTOXIC EFFECTS OF FE(III) NANOPARTICLES COMPARED TO FE(III) FINE PARTICLES IN HUMAN LUNG CELLS.

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In a comparative study the toxicity of nano- (<100 nm) and fine (<5 µm) hematite, arsenopyrite (<5 µm), TiO2 (<50 nm), and quartz (<5µm) particles were investigated. The physico-chemical analysis of the particles revealed a high content of Fe2O3 in hematite and arsenopyrite samples. DQ12 and TiO2 did not contain any iron. The arsenic content in the arsenopyrite sample was 16 ng/mg as measured by HPLC-ICP-MS. Uptake studies by transmission electron microscopy in human lung cells (BEAS-2B) showed that all particles were located close to the nuclear membrane and to mitochondria. No particles were found within the nucleus or mitochondria. Cyto- and genotoxicity studies in primary human lung fibroblasts (IMR-90) revealed that the hematite nanoparticles were most cyto- and genotoxic as assessed by trypan blue and comet assay. Arsenopyrite was negative in both of these test systems up to a tested concentration of 50 µg/cm2. Measurement of acellular radical generation by electron spin resonance indicated that fine hematite particles generated the highest amount of hydroxyl radicals in the presence of H2O2 compared to the hematite nanoparticles and TiO2. Treatment with the reducing agent ascorbic acid or cell lysates resulted in a high radical generation from the nanoparticles as compared to the fine particles. Intracellular formation of reactive oxygen species was measured by H2DCFDA staining and was found to be highest for fine hematite and lowest for hematite nanoparticles, arsenopyrite and TiO2. We conclude from our study that hematite nanoparticles may elicit DNA damage via a mechanism that involves a reduction and mobilisation of Fe(III) in the cytoplasm after cellular uptake. A direct interaction of Fe(II)/Fe(III) ions with DNA might also be a possible mechanism for induction of particle-induced genomic damage.

441 SINGLE-WALLED CARBON NANOTUBES: GENO- AND CYTO-TOXIC EFFECTS IN LUNG FIBROBLAST V79 CELLS.

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With the development of nanotechnology, there is a tremendous growth of the application of nanomaterials, which increases the risk of human exposure to these nanomaterials through inhalation, ingestion and dermal penetration. Among different types of nanoparticles, single walled carbon nanotubes (SWCNT) with extremely small size (1 nm in diameter) exhibit extraordinary properties and offer possibilities to create materials with astounding features. Since the release of nanoparticles in an enclosed environment is of great concern, a study of possible genotoxic effects is important. Our previous data showed that pharyngeal aspiration of SWCNT elicited pulmonary effects in C57BL/6 mice that were characterized by a robust, acute inflammatory reaction with an early onset and resulting in progressive interstitial fibrogenic response, and the formation of granulomas. In the present study, the genotoxic potential of SWCNT was evaluated *in vitro*. The genotoxic effects of nanoparticles were examined using three different test systems: the comet assay and micronucleus (MN) test in a lung fibroblast (V79) cell line, and the *Salmonella* gene mutation assay in strains YG1024/YG1029. Cytotoxicity tests showed loss of viability in a concentration- and time-dependent manner after exposure of cells to SWCNT. Results from the comet assay demonstrated the induction of DNA damage after only 3 hr incubation with 96 $\mu g/cm^2$ of SWCNT. The MN test indicated some but not significant micronucleus induction by SWCNT in the V79 cell line at the highest concentrations tested. With two different strains of *Salmonella typhimurium*, no mutations were found following SWCNT exposure.

442 A 28-DAYS REPEATED DOSE AND GENOTOXICTY STUDY OF SILVER NANOPARTICLES.

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Silver nanoparticle is one of the most controversial research areas regarding toxicity relative to biological systems. To evaluate the toxicity of silver nanoparticles, the rats (4 group, 20/group, male and female) were orally administrated once a day, for 28days at dosage level of 0, 30, 300 and 1,000 mg/kg/day. Mortality, clinical obser-

vations were made and body weight, water intake and food consumption were recorded weekly. At the end of study, the rats were subjected to necropsy, blood sample were collected for hematology, coagulation time assay, biochemistry parameters and silver content assay. To evaluate genetic toxicity, micronucleus test was also performed. All animals survived to the end of study. No test substance-related clinical signs and changes of body weight were observed. There were no dose-related changes in water intake, food consumption, urine volume, coagulation time, relative organ weight of all animals treated with silver nanoparticles. The micronucleus test using the bone marrows of the SD rats showed negative results. However, the hematology values of red blood cell, hemoglobin and hematocrit were increased in the 300 and 1,000mg/kg/day dose group in female rats. And the blood biochemical values of alkaline phosphatase, total cholesterol which indicated that the liver damage were increased in the 300 and(or) 1,000 mg/kg/day dose group in both sexes. In addition, histopathological findings revealed the evidence of hepatic injuries related to silver nanoparticle. Silver content assay showed dose-relative changes in brain, testis, lung in male rats and brain in female rats. Interestinlgy, the silver content of kidneys in the female rats was 2 times of those in the male rats. NOAEL of the test substance was considered to be less than 300 mg/kg/day for male and female rats under conditions in this study.

443 LIMITATIONS OF THE STANDARD *IN VITRO* GENOTOXICITY PROTOCOLS FOR TESTING OF NANOMATERIALS.

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Substances are usually tested as aqueous (or organic solvent) solutions in standard testing for genotoxic effects. Insoluble materials with at least two dimensions of less than 100 nm (nano- or ultrafine objects and particles) exhibit unique physical and chemical properties making them valuable for new technology. However, their unique physical properties may also enable direct interaction with DNA or trigger secondary mechanisms (e.g. oxidative stress) which can lead to genotoxic effects. This triggers the need to apply insoluble nanoparticles, rather than the usual dissolved test substances, to genotoxicity tests. We examined various modifications of the bacterial reverse mutation assay (OECD 471) and the in vitro chromosome aberration test (OECD 473) to apply insoluble nanomaterials to the test system. Various protocols to prepare dispersions from ten different nanomaterials were tested; methods and pitfalls in preparing and characterizing the dispersions are presented. In summary, the agglomeration state and the surface coating of particles in dispersion may vary with the surrounding fluid (water or DMSO, fetal calf serum, lung surfactant). The particle size distribution in the dispersions was analysed by light scattering and analytical ultracentrifugation. The methods and their limitations are evaluated. In general, the highest amounts of ultrafine particles were measured when using fetal calf serum. Our results in the bacterial reverse mutation assay, showed neither relevant bacteriotoxicity nor mutagenicity; in the in vitro chromosome aberration test precipitation of (agglomerated) particles on the slides interfered with scoring for chromosome breakage. Thus, extremely low concentrations have to be scored which are not expected to induce any toxic effect. The results indicate that preparation and characterization of the test dispersions is the critical step in applying standard genotoxicity tests to nanomaterials.

444 ALTU-237: A 14-DAY ORAL TOXICITY STUDY IN BEAGLE DOGS.

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ALTU-237 is an orally-deliverable crystalline formulation of an oxalate-degrading enzyme intended for use in patient populations with complications relating to hyperoxaluria, such as primary hyperoxaluria, enteric hyperoxaluria, and some types of recurrent calcium oxalate nephrolithiasis. The purpose of the current study was to determine the potential toxicity of ALTU-237 when administered for 14 consecutive days via oral gavage to male and female Beagle dogs. A total of 32 dogs (16/sex) were divided evenly into 4 groups (4/sex/group). Group 1 animals received vehicle (20mM Tris Buffer). Animals in Groups 2-4 received daily oral administration of ALTU-237 at doses of 20, 60, and 200mg/kg, respectively. Postdose evaluations of the animals included daily clinical observations, daily food consumption measurements, weekly body weight measurements, blood collection for clinical pathology assessment, and collection of a standard panel of organs at necropsy on study day (SD) 15 for histopathology evaluation. In addition, cardiovascular parameters (blood pressure, heart rate, EKG, respiration rate) and body temperature were recorded prior to study start and 2 hours postdose on SD 1. The results showed that daily treatment with ALTU-237 at doses up to 200 mg/kg/day had no effect on mortality, clinical and cageside observations, body weights, body weight changes, food consumption, blood pressure, heart rate, respiration rate, body temperature, electrocardiography, clinical pathology parameters, gross pathology, absolute and relative organ weights, or histopathology. In conclusion, administration of ALTU-237 at doses up to 200 mg/kg/day by oral gavage for 14 consecutive days to male and female beagle was well tolerated. The no-observed-effect-level (NOEL) under the conditions of this study is at least 200 mg/kg/day for 14 consecutive days.

445 MECHANISTIC ASPECTS OF PROPANIL NEPHROTOXICITY *IN VITRO*.

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Propanil, an agricultural herbicide, can induce nephrotoxicity in isolated rat renal cortical cells (IRCC). The purpose of the present study was to explore the role of metabolites and oxidative stress in propanil nephrotoxicity in vitro. IRCC were obtained from male Fischer 344 rats and incubated in Krebs-Henseleit buffer (pH = 7.37) at 37 degrees C under a 95% oxygen/5% carbon dioxide atmosphere with shaking. One of a selection of pretreatments or pretreatment vehicle was added (30 μl) prior to the addition of propanil (1 mM final concentration). Following propanil addition, incubations were continued for 120 min. Cytotoxicity was determined by measuring lactate dehydrogenase (LDH) release (% of total). Propanil cytotoxicity was reduced by piperonyl butoxide, but not by ascorbate, α-tocopherol, deferoxamine, glutathione, indomethacin, methimazole, N-octylamine, isoniazid or metyrapone. These results indicate that propanil nephrotoxicity may result from a metabolite formed by cytochrome P450 (possibly CYP1A family) oxidation of propanil, but oxidative stress or reactive metabolites do not appear to contribute to propanil nephrotoxicity in vitro. (Supported by NIH Grant 5 P20 RR016477 to the West Virginia IDeA Network for Biomedical Research Excellence.)

446 DETECTION OF ENDOTOXIN-INDUCED ACUTE KIDNEY INJURY IN RATS.

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Sepsis, a serious medical condition that affects many patients in the ICU, is a leading cause of acute kidney injury (AKI). Administration of lipopolysaccharide (LPS) can be used to produce many of the inflammatory and multiple target organ effects that occur in sepsis. The objective of this study was to determine if AKI can be detected in LPS-treated rats using urinary biomarkers that have been shown to detect AKI in rats exposed to prototypic nephrotoxic agents. Male rats were administered a single injection of *E. coli* LPS (0.5, 1, 2, or 3 mg/kg, ip); controls received saline. Immediately after injection, rats were placed in metabolism cages for either a 4- or 18-hr urine collection, after which the animals were weighed, a rectal temperature was obtained, and blood and kidneys were removed for analysis. After the 4-hr exposure, animals treated with even the lowest dose of LPS exhibited signs of endotoxemia, including weight loss, hypothermia, and granulocytosis, compared to controls. Levels of urinary N-acetyl-β-D-glucosaminidase (NAG) were elevated and urine specific gravity was decreased at all LPS doses, while no changes were evident for urine volume, urinary kidney injury molecule-1 (Kim-1), and blood urea nitrogen (BUN) and creatinine. After 18-hr exposure, BUN and urinary Kim-1 were elevated at the highest LPS doses, urine volume and specific gravity were decreased, and no changes were observed in NAG or blood creatinine. The results show that urine NAG elevation and decreased urine specific gravity can be detected within the first 4 hours of an endotoxemic response in rats. At the same time, other markers of renal injury are not altered, including changes in serum creatinine, an endpoint that currently serves as a "gold standard" for assessing renal damage in preclinical and clinical studies. AKI can be detected after 18 hours exposure to LPS by increases in urine volume, BUN, and urinary Kim-1, without increased levels of blood creatinine.

447 ROLES OF MITOCHONDRIAL DYSFUNCTION, CASPASES, AND FAS RECEPTOR IN S-(1, 2-DICHLOROVINYL)-L-CYSTEINE (DCVC)-INDUCED CYTOTOXICITY IN HUMAN PROXIMAL TUBULAR (HPT) CELLS.

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To investigate whether mitochondrial dysfunction is required for DCVC-induced nephrotoxicity or whether cell death can also occur by mitochondria-independent pathways, the influence of mitochondrial inhibitors on responses of primary cultures of hPT cells to DCVC exposure was determined. DCVC-induced changes in

mitochondrial functions were compared to time- and concentration-dependent effects on necrosis, apoptosis, and cell proliferation status. DCVC (10-500 μM) produced time- and concentration-dependent inhibition of cellular oxygen consumption that reached ~90% inhibition with 500 μM DCVC at 48 hr exposure. DCVC produced a modest amount of apoptosis at relatively low concentrations (50 μM), which was prevented by cyclosporin A (CsA). DCVC-induced depolarization of the mitochondrial membrane potential was diminished by CsA but was exacerbated by the peripheral benzodiazepine receptor antagonist PK11195. Unlike other oxidants such as tert-butyl hydroperoxide, which produced more than 80% ATP depletion at 50 μM, DCVC only produced modest decreases in cellular content of ATP; concentrations of 300 μM or more were required to deplete ATP by more than 50%. DCVC increased activity of caspase-3/7 at early times (4 hr) and relatively low concentrations (50 μM) but had little effect on activity of caspase-8. Activation of caspase-3/7 is linked to the mitochondrial (intrinsic) pathway of apoptosis whereas activation of caspase-8 is linked to the Fas receptor (extrinsic) pathway. Unlike lipopolysaccharide, which is known to cause apoptosis in renal proximal tubular cells in part by a Fas receptor-dependent, mitochondria-independent pathway, DCVC had no effect on expression of Fas receptor. These results are consistent with previous data showing mitochondria are a prominent and early target for DCVC and that production of mitochondrial dysfunction is an obligatory step in the various responses of hPT cells to DCVC. Moreover, DCVC does not appear to produce apoptosis by the Fas receptor-dependent pathway.

448 MITOCHONDRIAL GLUTATHIONE TRANSPORT IN DIABETIC NEPHROPATHY.

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Nephropathy is a serious and prevalent complication of diabetes, and is associated with oxidative stress and altered mitochondrial function. In previous work, we defined the function of two inner membrane carriers, the 2-oxoglutarate carrier (OGC) and dicarboxylate carrier (DIC), as being responsible for transport of glutathione (GSH) into renal mitochondria. To study the importance of mitochondrial function and GSH transport in diabetes and to develop a protective strategy against the associated nephropathy, a diabetic rat model was established by ip injection of streptozotocin (STZ) into male Sprague-Dawley rats. Two in vitro systems were used, suspensions of isolated mitochondria from renal cortex and primary cultures of proximal tubular cells. We test the hypothesis that diabetic nephropathy is characterized by mitochondrial dysfunction and decreased rates of GSH transport, and then assess the ability of over¬expression of mitochondrial GSH carriers to revert the diabetic nephropathy phenotype to normal. As a first step, we characterized mitochondrial function and GSH and dicarboxylate transport in isolated mitochondria from normal and STZ-induced diabetic rats one month post-injection (i.e., before the onset of nephropathy). Even at this early stage of diabetes, rates of succinate-linked State 3 respiration, respiratory control ratios, activities of GSH peroxidase, GSH S-transferase, and malic dehydrogenase, and intramitochondrial concentrations of GSH were significantly higher in mitochondria from diabetic rats as compared to those from control rats. In contrast, kinetics of GSH and dicarboxylate transport were only slightly altered by diabetes. Mitochondria from diabetic rats, however, were markedly more susceptible than those from normal rats to oxidative injury. Primary cultures of proximal tubular cells from diabetic rats exhibit modest cellular hypertrophy, which may be responsible for the changes in energetics and redox status. Studies will next be pursued at later stages of diabetes, when nephropathy develops.

449 PUROMYCIN TREATMENT RESULTED IN DIFFERENTIAL EXPRESSION OF GENES INVOLVED IN PATHWAYS ASSOCIATED WITH GENE/PROTEIN EXPRESSION PROCESS.

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Puromycin dihydrochloride (PUR), which inhibits protein synthesis, is a commonly used model nephrotoxin that results in glomerular injury. To gain further insights into the mechanism of PUR nephrotoxicity, gene expression changes in the kidney tissues isolated from rats 7 days after PUR treatment (5 - 150mg/kg) were studied using Affymetrix RAE230A Genechip arrays. The combined result of statistical analyses revealed that PUR exposure resulted in differential expression of 211 genes. Self-Organizing Map clustering analysis identified three gene clusters with unique dose response patterns of progressive up-regulation, down-regulation and a U-shaped pattern of 114, 76 and 21 genes, respectively. Gene ontology and pathway analyses revealed that the genes involved in cellular processes such as cell organization and biogenesis, DNA metabolism and packaging, protein synthesis and metabolism, and mRNA transcription and processing are over-represented among the differentially expressed genes. As PUR is a potent inhibitor of protein synthesis, up-regulation of genes involved in mRNA transcription and processing, and protein synthesis and metabolism likely constitutes a cellular defense mechanism to counter PUR toxicity. Interestingly, four genes (Atp2a2, Camk2d, Gnb2 and Ywhaq) involved in the pathway of calcium regulation of cardiac cells were differentially expressed, as revealed by GenMAPP analysis. The functions of these genes range from calcium signaling and transport, intracellular signal transduction, cell cycle regulation, and DNA repair. Although several of these genes have been reported to play a role in renal functions/injuries, the significance of calcium regulation in PUR-induced glomerular injury and/or cellular response to this insult however has yet to be elucidated.

450 ATTENUATION OF N-(3, 5-DICHLOROPHENYL)-2-HYDROXYSUCCINIMIDE-O-SULFATE (NSC)-INDUCED NEPHROTOXICITY IN PRIMARY CULTURES OF RAT RENAL PROXIMAL TUBULE CELLS.

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The agricultural fungicide N-(3,5-dichlorophenyl)succinimide induces nephrotoxicity via metabolites that arise from oxidation of the succinimide ring. Recent studies have shown that one metabolite, N-(3,5-dichlorophenyl)-2-hydroxysuccinimide-O-sulfate (NSC), may be a penultimate or ultimate nephrotoxic species. The purpose of this study was to examine the nephrotoxic potential of NSC and to determine pretreatments that could attenuate NSC nephrotoxicity using primary cultures of rat renal proximal tubule cells (RPTC) obtained from male Fischer 344 rats. Renal proximal tubular segments were isolated following collagenase perfusion of the kidney, enriched by differential centrifugation and plated (0.5 mg protein) on plastic dishes to grow RPTC. Cells were cultured for 5 days (~90-95% confluent) and then the primary cultures treated with NSC (1 mM) or vehicle and incubations continued for up to 5 hr. In some experiments, a pretreatment or pretreatment vehicle was added prior to NSC or NSC vehicle and incubations continued for 3 hr post NSC or NSC vehicle. Cytotoxicity was measured as an increase in lactate dehydrogenase (LDH) release (% of total). NSC alone induced cytotoxicity by 3 hr. Pretreatment with 1 mM N-acetylcysteine, glutathione or alanine significantly decreased NSC cytotoxicity. Pretreatment with 5 mM or more p-aminohippurate resulted in a small, but significant, concentration-dependent decrease in NSC cytotoxicity, while pretreatment with 1 mM probenecid or glycine did not affect NSC cytotoxicity. These results suggest that NSC induces cytotoxicity via a reactive metabolite and may enter RPTC via a probenecid-insensitive transporter. Supported in part by NIH grant DK31210.

451 URINARY KIDNEY INJURY MOLECULE-1 AND N-ACETYL β-D-GLUCOSAMINIDASE AS BIOMARKERS FOR AMINOGLYCOSIDE AND CISPLATIN-INDUCED NEPHROTOXICITY IN PATIENTS.

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The objective of this study was to evaluate the sensitivity and specificity of kidney injury molecule-1 (KIM-1) and N-acetyl-β-D-glucosaminidase (NAG) as biomarkers of nephrotoxicity (AKI) in patients receiving aminoglycosides or cisplatin. Urine was collected prior to and then daily following tobramycin or amikacin administration to patients with cystic fibrosis receiving a 14-day course for acute pulmonary exacerbations (N = 23). Three patients that developed AKI (rise in SCr > 0.3 mg/dL), both urinary KIM-1 and NAG increased over two-fold prior to or coincident with the rise in serum creatinine (SCr). KIM-1 levels at baseline were within the normal range (as assessed in healthy volunteers) and increased in 5 of 20 patients without an increase in SCr > 0.3 mg/dL. By contrast NAG levels at baseline prior to treatment were significantly higher than "normal". NAG rose more than two-fold in virtually every patient (20 of 23; median increase 5.1 fold, 10th and 90th percentiles 0.8-76 fold) with (3 of 3) or (17 of 20) without AKI raising the possibility of non-specificity. In contrast, only eight of 23 patients had a greater than two-fold increase in urinary KIM-1 (median increase 1.3-fold, 10th and 90th percentiles 0.5 – 9-fold).

Urine samples were obtained prior to the first dose and then following each cisplatin dose (20 mg per m2 infused over 1 hour) in men receiving 4 cycles of outpatient-based chemotherapy for testicular cancer (N = 10). Both KIM-1 and NAG increased in parallel over the five days of a given cycle of chemotherapy and then returned towards baseline by the beginning of the subsequent cycle. In no patient was SCr, an insensitive marker for nephrotoxicity, increased.

Thus, KIM-1 and NAG both appear to be sensitive biomarkers for early detection of clinical nephrotoxicity although the specificity of NAG requires further evaluation.

452 MODULATION OF OXIDATIVE STRESS BY RESVERATROL AND ATTENUTATION OF CISPLATIN *IN VITRO* RENAL TOXICITY.

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Cisplatin is a cancer chemotherapeutic agent which is associated with irreversible renal toxicity. The development of interventions to delay or prevent the cisplatin associated renal toxicity would be of direct clinical relevance. Resveratrol is a naturally occurring phytochemical found in grapes, nuts and other foods. Resveratrol has been reported to possess some anticancer and also antioxidant properties. The following study investigated whether resveratrol would reduce the in vitro renal toxicity of cisplatin in a renal cortical slice model. Male Fischer 344 rats weighing 200-250 g were used in all studies. Rats were anesthetized with isoflurane and the kidneys were isolated, decapsulated and rinsed in 3 ml ice cold Krebs buffer. Renal cortical slices were prepared and 50-100 mg of tissue was pre-incubated with ethanol (VEH) or 30 ug/ml resveratrol (RES, final concentration) for 30 min at 37oC under an oxygen atmosphere. The tissue were next incubated in 3 ml oxygenated Krebs containing 0, 75, or 150 ug/mL cisplatin for 120 min under an oxygen atmosphere and constant shaking at 37oC. Loss of membrane integrity was evaluated by measurement of lactate dehydrogenase (LDH) leakage following the 120 min incubation. Total renal glutathione and lipid peroxidation were measured along with western analysis to evaluate the effect of resveratrol on cisplatin mediated oxidative stress. Lipid peroxidation was increased ($p<0.05$) by 75 and 150 ug/ml cisplatin relative to VEH values. Pre-incubation with RES for 30 min blocked the cisplatin mediated rise in lipid peroxidation. Pretreatment with RES had no effect on cisplatin mediated decline in total renal glutathione levels. Cisplatin increased protein cabonyls and 4-hydroxynonenal adducted proteins as observed following Western blot analysis. In summary RES diminished cisplatin renal toxicity in vitro and reduced cisplatin mediated oxidative stress. (Supported by NIH Grant COBRE 5P20RR020180).

453 GENE EXPRESSION ANALYSIS REVEALS NEW POSSIBLE MECHANISMS OF VANCOMYCIN-INDUCED NEPHROTOXICITY.

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Vancomycin, one of few effective defenses against methicillin-resistant Staphylococcus aureus, is nephrotoxic. The goals of this study were to gain insights into molecular mechanisms of nephrotoxicity induced by vancomycin at the genomic level and identify potential gene expression markers of vancomycin-induced renal injury. Groups of 6 female BALB/c mice were treated with 7 consecutive daily intravenous (iv) or intraperitoneal (ip) doses of vancomycin (50, 200, or 400 mg/kg) or saline, and sacrificed on Day 8. Changes in clinical chemistry, such as a significant increase in blood urea nitrogen (BUN), were observed in mice treated with 400 mg/kg vancomycin. Histopathologic evaluation also indicated renal injuries at the 400 mg/kg dose level in both oral and iv groups. Hierarchical clustering analysis revealed that kidney gene expression profiles of all mice treated at 400 mg/kg clustered together and resembled the profiles in mice administered 200 mg/kg iv, suggesting that transcription profiling might be more sensitive than current clinical markers for detecting kidney damage. Analysis of transcripts whose expression was changed at least 2-fold after administration of high doses of vancomycin compared with the vehicle group confirmed the implication of oxidative stress and impaired mitochondrial energy dependent transport system as a mechanism of vancomycin-induced toxicity. In addition, our analysis revealed significant changes in the complement and inflammatory transcripts suggesting vancomycin nephrotoxicity might depend on inflammation and complement pathway activation, similar to the mechanism of injury after renal ischemia/reperfusion. Supported by NIAID Contract N01-AI-05417.

454 CHARACTERIZATION OF PROPIVERINE EFFECTS ON RAT PPARS.

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Propiverine is the active ingredient of Mictonorm® (Apogepha, Germany), a well-tolerated drug prescribed for the treatment of symptoms of an overactive bladder. Recent in vivo studies (Heussner et al., 2006) demonstrated a reversible accumulation of D-amino acid oxidase (DAAO) in rat proximal tubular cells following 16 weeks of dietary propiverine exposure. Species comparison suggested that rat renal specific DAAO expression and accumulation is under peroxisomal proliferator-activated receptor (PPAR) and RXR (retinoid X receptor) control. Therefore, the objective of the ensuing experiments was to study the involvement of PPARs and RXRs in the above propiverine-induced rat-specific pathology using an in vivo and in vitro approach. Kidney and liver samples from the in vivo study, as well as samples from sub-chronically propiverine- / propiverine-N-oxide-exposed rat renal continuous cells (NRK-52E) were analyzed for altered expression of DAAO, all three subtypes of PPAR and RXR at the mRNA level, using RT-PCR, with subsequent electrophoresis and densitometry. Preliminary data demonstrated an increased expression of PPARα and β as well as RXRα and γ following in vivo exposure to propiverine. A similar trend was suggested in NRK52E cells following exposure to propiverine- / propiverine-N-oxide. Using DAAO, PPAR α and β and RXR-specific antibodies in a Western analysis confirmed the mRNA data for PPAR α and β and DAAO, thus supporting the assumed involvement of PPARs in the propiverine-induced accumulation of DAAO in rats.

455 THE POTENTIAL EFFECT OF MT-AB ON RENAL DYSFUNCTION WITHIN A CHINESE DIABETIC POPULATION.

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It has been reported the presence of metallothionein antibody (MT-Ab) increased the susceptibility for tubular damage among cadmium workers. This study focused on the relationships between levels of MT-Ab, urinary cadmium, and kidney function in a Chinese type 2 diabetic population. A cross-sectional study was performed on 229 type 2 diabetic patients (92 men and 137 women), who were recruited from two nearby communes s in the same district of Shanghai, China. Information was obtained from interviews, health records, blood and urine samples.
Levels of the r β2-microglobulin increased significantly when the levels of MT-Ab and urinary cadmium were elevated in male and female subjects; in contrast, the levels of urinary albumin, did not display such a pattern. After adjusting for potential confounding covariates, logistic regression showed that the odds ratios (ORs) of tubular dysfunction increased upon 1) increasing the MT-Ab concentration from a low to high level (OR 5.56 [95% CI 2.25–13.73]) and 2) increasing the level of urinary cadmium from <1 to 1≧g/g creatinine (3.34 [1.17–9.53]); the OR of patients currently smoking was 3.51 [1.14 –10.80] relative to that of those who had never smoked.
This study demonstrated that the presence of MT-Ab could potentiate tubular dysfunction among diabetic subjects and that patients with high MT-Ab levels are more prone to development of tubular damage.
Key words: diabetes; metallothionein; metallothionein antibody; renal dysfunction; cadmium

456 CHANGES IN THE RENAL EXPRESSION OF RPA-1, RPA-2, AND KIM-1 FROM SPONTANEOUSLY HYPERTENSIVE RATS (SHR) GIVEN DOXORUBICIN (DXR) OR MITOXANTRONE (MTX), WITH OR WITHOUT DEXRAZOXANE (DRZ).

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Our previous studies have shown that the antineoplastic agents DXR and MTX induced cardiorenal injury in SHR and that DXR-induced nephrotoxicity was more severe than that resulting from MTX. The cardiorenal toxicity can be attenuated by pretreatment with DRZ, an intracellular iron chelator. Cardiac troponin T has been shown to be a sensitive biomarker of DXR- or MTX-induced cardiac injury; however, potential biomarkers of the nephrotoxicity induced by these two agents have rarely been addressed. The present study was undertaken to determine whether renal expression of RPA-1 in collecting ducts, RPA-2 in the loop of Henle and Kim-1 in the proximal convoluted and straight tubules may be changed in response to DXR- or MTX treatment and as a result serve as potential renal biomarkers. SHR were given 1 mg/kg DXR (iv) or 0.5 mg/kg MTX (iv), with or without pretreatment with 25 mg/kg DRZ (ip) once a week for 10-12 weeks. Immunostaining of renal tissue from DXR- or MTX-treated rats showed (1) RPA-1, RPA-2, and Kim-1 expression was increased in distinct segments of the nephron compared with that observed in kidneys of SHR given saline or DRZ; (2) changes in expression were greater in DXR-treated than in MTX-treated rats; (3) increases in RPA-1, RPA-2, and Kim-1 expression were less in SHR pretreated with DRZ and (4) changes in the levels of RPA-1, RPA-2, and Kim-1 expression correlated with the severity of renal lesion scores (as assessed by light microscopic examination based on

a semiquantative scoring scale of 0-4). These findings suggest that the renal alterations induced by DXR or MTX in SHR are complex and that proteins such as Kim-1, RPA-1 and RPA-2 may serve as biomarkers to identify specific sites of nephron injury in this model.

457 SPONTANEOUS OCCURENCE OF A DISTINCTIVE AMPHOPHILIC-VACUOLAR RENAL TUBULE TUMOR PHENOTYPE IN RAT CARCINOGENICITY STUDIES CONDUCTED BY THE NATIONAL TOXICOLOGY PROGRAM.

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Carcinogenicity studies, utilizing primarily the Fischer 344 rat, conducted by the National Toxicology Program, NIEHS, NIH were reviewed for the occurrence and distribution of a distinctive renal tubule tumor phenotype. Both control and treated groups were examined. The main features of this tumor included the uniform lobular arrangement of eosinophilic to amphophilic stained, large round to polyhedral cells with finely granular cytoplasm and the presence of numerous variably-sized clear vacuoles and/or minilumens. Central degeneration/necrosis was also prominent in most cases. It is referred to as the "Amphophilic-Vacuolar" renal tubule tumor to help distinguish it from the predominant rat renal tumors which are basophilic staining. Out of 1012 rats surveyed with renal tumors (adenomas, carcinomas or adenocarcinomas), 100 of these had the amphophilic-vacuolar morphology. In a few cases, tumors were multiple and bilateral. These tumors were equally distributed between sexes, did not metastasize, at least to the lungs, and did not appear to be associated with areas of chronic progressive nephropathy.
The distribution of this tumor was random across studies and dose groups suggesting it was spontaneous and not chemically induced. Results from this review and other published reports of similar appearing tumors, particularly from young and possibly sibling rats, indicate that this tumor may be of familial origin. If this hypothesis can be validated then tumors of this phenotype could be excluded from final tumor counts when assessing the carcinogenic potential of test chemicals in preclinical studies. This tumor will be briefly compared to Eker and Nihon rats which are familial renal cancer models. The tumor phenotype closely resembled one of two phenotypes commonly observed in the Eker rat.

458 UPREGULATION OF GLYCERALDEHYDE 3 PHOSPHATE DEHYDROGENASE (GAPDH) AND GLUCOSE 6 PHOSPHATE DEHYDROGENASE (G6PDH) DURING THE EARLY STAGES OF CADMIUM-INDUCED RENAL INJURY.

J. Edwards, P. C. Lamar and W. C. Prozialeck. *Pharmacology, Midwestern University, Downers Grove, IL.*

Exposure to cadmium (Cd) results in injury to proximal tubule epithelial cells as indicated by a variety of endpoints such as: oxidative stress, disruption of cell adhesion molecules and alterations in cell cycle control. Although some of the generalized cellular responses to Cd-induced injury are well documented, such as up-regulation of metallothionein; other cellular responses are just now being elucidated. In this study, we show that G6PDH and GAPDH are up-regulated in a subchronic animal model of Cd-induced nephrotoxicity at a time point before overt renal dysfunction appears. Male Sprague/Dawley rats were given daily subcutaneous injections of 0.6 mg/kg of Cd in the form of $CdCl_2$ for up to 12 weeks and weekly urine samples were analyzed for markers of renal dysfunction. After 3, 6 and 12 weeks, representative animals were sacrificed and samples of kidney were analyzed for oxidative stress (TBARS) and expression of GAPDH and G6PDH by Western blot analysis. After 3 weeks of Cd treatment, there were no apparent differences in expression of G6PDH or GAPDH, but at 6 weeks, expression of renal GAPDH and G6PDH was increased by approximately 2 fold. In this model, increases in traditional urinary markers of Cd exposure such as metallothionein, Clara cell protein-16 and Cd occur at later time points (9-12 weeks). There was no significant change in TBARS at 6 weeks of Cd exposure. At 12 weeks, the increase in GAPDH and G6PDH expression was even more pronounced; there was also a significant increase in TBARS at this time point. GAPDH and G6PDH produce NADH and NADPH, respectively, and play key roles in cellular thiol metabolism. The fact that these enzymes were up-regulated at a time point before the onset of Cd-induced renal dysfunction and overt signs of oxidative stress, may indicate that they are "sentinel" proteins and part of an early cellular defense and/or stress response. Supported by Grant RO1-ES006478 from the NIEHS.

459 THE QUANTITATIVE DETERMINATION OF GENTAMICIN-, INDOMETHACIN- AND AMPHOTERICIN-INDUCED RENAL INJURY USING AN ETHIDIUM HOMODIMER-BASED *IN SITU* VIABILITY ASSAY.

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Recently, we reported a new method to quantify proximal tubule epithelial cell viability that involves perfusing the intact rat kidney with ethidium homodimer. We wanted to determine if this new technique could be used to characterize toxic injury in the three main areas of the kidney; the cortex, medulla and papillus. To do this, rats (n = 6 per group) were treated with the site-specific nephrotoxicants, gentamicin (proximal tubule/cortex; 100 mg/kg i.p. per day for 8 consecutive days), indomethacin (papillus; 20 mg/kg oral gavage, single dose) and amphotericin B (distal tubule/medulla; 15 mg/kg i.p. per day for 5 days). At the end of the respective treatment periods, ethidium homodimer (5 μM) was infused (3 ml/min for 10 min) into the left intact kidney while the animal was anesthetized. The kidney was then perfused with a physiologic saline solution, removed, placed in embedding medium, frozen, cryosectioned at a thickness of 5μm and ethidium-labeled cells counted. The gentamicin-treated animals exhibited significant ($p < 0.05$) polyuria and proteinuria with no change in urinary creatinine excretion. Indomethacin- and amphotericin-treated animals did not show significant differences in either urine volume or urinary protein, but did show significantly lower urinary creatinine levels. Gentamicin treatment resulted in 15.4 ± 3.0% of the cells located in the cortex to be positively labeled for ethidium homodimer. Renal cell death resulting from indomethacin and amphotericin treatment was localized in the papillus (8.2 ± 1.3%) and medulla (8.8 ± 2%), respectively. Percent ethidium homodimer labeling in all areas of all control animals was around 1%. These results show that this in situ viability assay provides a sensitive and accurate way to identify cell death in the three main areas of the kidney following exposure to site-specific nephrotoxicants. Supported by Grant RO1-ES006478 from the NIEHS.

460 PATHWAYS OF ρ-NONYLPHENOL (NP)-INDUCED RENAL TOXICITY SUGGESTED BY GLOBAL GENE EXPRESSION PROFILES.

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NP, widely used in the manufacture of nonionic surfactants, lubricant additives and polymer stabilizers, is a putative endocrine disruptor due to its weak estrogenic activity and induces renal toxicity by an undetermined mechanism. Our previous studies have shown that NP induces polycystic kidney disease (PKD) in Sprague-Dawley rats exposed to 2000 ppm NP from the time of organogenesis (gestation day 7) until termination at postnatal day (PND) 50, and that dietary components in rodent diets modulate this toxicity. To gain insights into the molecular mechanisms by which NP induces PKD and into the protective pathways modulated by diet, we analyzed changes in the kidney gene expression profiles by microarray technology. Male Sprague-Dawley rats were placed on a PKD-permissive chow (low phytoestrogen Purina 5K96) or a PKD-protective chow (high phytoestrogen Purina 5001) supplemented with 0 or 2000 ppm NP, from gestation day 7 until PND 21. As previously reported, a higher apoptotic index in renal tubules is found at PND 21 in rats fed NP in 5K96 diet, although PKD is not evident at this time. In-house printed microarrays composed of 6000 known rat genes were used in a 2-color reference design experiment. Analysis of the resulting data by Welch t-test and fold-change revealed that NP altered the expression of 120 genes in rats fed with the low-phytoestrogen chow and only of 61 genes in rats fed with the high-phytoestrogen chow. Several of the genes modulated by NP in combination with the low-phytoestrogen diet, but not with the high-phytoestrogen diet, are associated with intracellular calcium homeostasis (voltage-gated calcium channels and calmodulin 2 genes), as well as with signalling involving Wnt (Wisp2 gene) and cyclic AMP (PKA-1 gene), mechanisms known to play a role in the pathogenesis of polycystic kidney disease. This research was supported in part by the Oak Ridge Institute for Science and Education.

461 ZINC SUPPLEMENTATION PARTIALLY PREVENT DIABETIC NEPHROPATHY.

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Oxidative stress is a major pathogenic factor to diabetic nephropathy, and overexpression of metallothionein (MT) as a potent antioxidant in the heart significantly protected from diabetic cardiomyopathy. The present study aimed to examine

whether zinc (Zn) supplementation induces renal MT synthesis and protects from diabetic nephropathy. Diabetic rats were induced with streptozotocin (STZ) and then the hyperglycemic rats were treated either with Zn supplementation or saline for three months. Diabetic nephropathy was examined by renal function and pathological alterations. Results showed that Zn supplementation immediately given after the onset of hyperglycemia in STZ-diabetic rats for three months significantly prevented the development of renal dysfunction and pathological changes. Zn supplementation also significantly prevented diabetes-induced renal oxidative damage and up-regulated expression of pro-fibrosis mediators connective tissue growth factor (CTGF) and plasminogen activator inhibitor 1 (PAI-1), along with a significant increase of renal MT synthesis predominantly in the tubular cells. Furthermore, direct exposure of renal tubular (HK11) cells to high levels of glucose (HG) induced CTGF up-regulation predominantly through ERK1/2-dependent, and partially through p38 MAPK-dependent pathways. Zn and cadmium pretreatment of HK11 cells both significantly induced MT expression, and suppressed HG-induced CTGF expression. Zn-induced MT synthesis and protection from HG-induced CTGF were not affected by p38 MAPK inhibitor, but partially affected by ERK1/2 inhibitor. This study provides the first evidence that Zn supplementation can partially prevent diabetic nephropathy through renal MT induction (Supported in part by research grants from ADA).

462 IDENTIFICATION OF A RAT SPECIFIC MECHANISM FOR ANTISENSE OLIGONUCLEOTIDE (ASO) INDUCED KIDNEY INJURY.

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Glomerular proteinuria [protein to creatinine (P/C) ratio >10] is associated with a small subset of ASOs such as ISIS 104838 in rat. This sequence-specific effect is unique to rat kidney and has not been observed in mouse, monkey or human. After a single subcutaneous injection of 500 mg/kg ISIS 104838 on Day 1, the plasma Cmax was reached after 3 hours, with a continuous low level ASO exposure in plasma up to 3 days post-dose. Glomerular proteinuria was developed between Day 16-21 with P/C ratio > 50. Increasing duration of daily serine protease inhibitor (AEBSF) treatment up to Day 9 has shown incrementally progressive inhibition of proteinuria, with up to ~ 90% reduction of urine protein (P/C = 4.6) on Day 21. This strongly implicates the causative role of protease mechanism, since no morphological changes of glomerulus and no proteinuria were observed at that time. The activity of circulating proteases is regulated by various endogenous protease inhibitors such as α 2-macroglobulin (A2M). ASOs bind to A2M, and it is possible an ASO-protease inhibitor (A2M) interaction in vivo leads to an alteration in inhibitory capacity. Preliminary data has shown a transient decrease in plasma A2M level (40%) during the peak hours of ASO exposure, which also correlated with a moderate elevation of protease activity on Day 2-7 after ASO treatment. The total protease inhibitor capacity has also been investigated using serum from mouse, rat, monkey and human to address the species specificity. Total protease activity was measured after adding increasing amounts of trypsin in the serum. Rat serum has shown the most dramatic increases of protease activity after trypsin challenges, indicating a lower protease inhibitor capacity than other species. Current data strongly suggest the causative role of serine proteases in ISIS 104838-induced kidney injury, and the low endogenous protease inhibitor capacity in rat could make it more susceptible to ASO-induced proteolytic injury in kidney.

463 THE POTENTIAL ROLE OF THE SODIUM IODIDE SYMPORTER IN BROMATE-INDUCED KIDNEY CELL DEATH AND DNA DAMAGE.

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The mechanism of bromate (BrO_3^-)-induced toxicity in Normal Rat Kidney cell lines after acute exposure to high concentrations was investigated. BrO_3^-, (0-1,600 μg/mL; added as $KBrO_3$) induced time- and concentration-dependent decreases in 3-(4, dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) staining after 24 and 48 hr. BrO_3^- also increased phosphatidylserine externalization and decreased membrane integrity after 48 hr as assessed by annexin V and PI staining. Cell cycle analysis demonstrated that BrO_3^- (200 and 400 μg/mL) induced a G2/M arrest after 48 hr in tandem with increases with the appearance of a Sub-GO/G1 peak, suggesting the hypothesis that BrO_3^- induces DNA damage. In support of this hypothesis BrO_3^- induced time- and concentration-dependent increases in nuclear fragmentation as assessed by the staining of 4',6-diamidino-2-phenylindole and fluorescence microscopy. Nuclear fragmentation was increased after 24 hr, and occurred in the absence of decreases in MTT staining, alterations in annexin V and PI staining or cell morphology. To investigate the role of the sodium iodide symporter (NIS) in the preferential transport of BrO_3^- into the kidney $NaClO_4$ (20 mM), a NIS inhibitor, was used to evaluate kidney cell death and DNA damage. Treatment of cells with $NaClO_4$ prior to exposure to BrO_3^- preserved cell morphology and MTT staining, compared to cells exposed to BrO_3^- only. Treatment with $NaClO_4$ prior to BrO_3^- treatment also reduced nuclear fragmentation compared to cells exposed to BrO_3^- only. These data suggest that BrO_3^--induced DNA damage occurs prior to phosphatidylserine externalization, loss of membrane integrity and cell death. Further, these data suggest a role for the NIS in BrO_3^--induced kidney cell death. This work was supported by the AwwaRF Project 4042 and the Georgia Cancer Coalition.

464 CADMIUM INHIBITS LACTATE GLUCONEOGENESIS IN HUMAN RENAL PROXIMAL TUBULES.

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Cadmium is a nephrotoxic heavy metal that disturbs the function of the renal proximal tubule, but it remains unknown if it interferes with the metabolism of the latter nephron segment. Given the importance of lactate as a physiological substrate of the human kidney in vivo, we have conducted an in vitro study to characterize the effect of cadmium on lactate metabolism.

Isolated human proximal tubules were incubated with unlabeled or variously 13C-labeled lactates or lactate plus 13C-bicarbonate. After incubation, substrate removal and product formation were measured by enzymatic and 13C MRS methods.

CdCl2 inhibited gluconeogenesis from 1 mM lactate in a dose-dependent manner with a IC50 of 0.1 mM. The labeling patterns of the glucoses synthesized from [1-13C]-, [2-13C]- and [3-13C]-lactate as well as from lactate plus 13C-bicarbonate indicated that lactate gluconeogenesis involved not only the passage of carbons through pyruvate carboxylase and the reversible equilibration of oxaloacetate with fumarate but also pyruvate dehydrogenase and the entire tricarboxylic acid cycle. Combination of enzymatic and MRS methods with a mathematical model of lactate metabolism, which provides a panoramic view of lactate metabolism, reveals that 0.1 mM CdCl2 inhibited lactate removal and fluxes through pyruvate carboxylase, phosphoenolpyruvate carboxykinase and the subsequent steps of lactate gluconeogenesis. The cellular level of acetyl-CoA level was also diminished by 0.1 mM CdCl2. By contrast, 0.1 mM CdCl2 did not alter fluxes through pyruvate dehydrogenase and through the entire tricarboxylic acid cycle although it significantly decreased the cellular coenzyme A and ATP levels.

It is concluded that cadmium strongly inhibits human renal lactate gluconeogenesis, an effect consistent with the fall of the cellular levels of not only acetyl-coenzyme A, a cofactor of pyruvate carboxylase, but also of ATP whose mitochondrial synthesis appears to be inhibited.

465 METHYL ISOBUTYL KETONE (MIBK) INDUCED α2u-GLOBULIN (α2u) NEPHROPATHY IN MALE AND FEMALE F344 RATS.

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Chronic MIBK exposure caused an increased incidence of renal tubule hyperplasia and renal tubule adenomas, and occurrence of renal tubule carcinomas in male rats only. A number of chemicals have been shown to cause male rat renal tumors through the α2u-nephropathy-mediated mode of action (MOA). The objective of this study was to investigate the MOA of MIBK induced renal tumors in male rats by evaluating the ability of MIBK to induce measures of α2u-nephropathy in male and female rats compared with that of d-limonene, a chemical characterized as a known inducer of α2u-nephropathy. Male F344 rats were administered by oral gavage corn oil (vehicle control), d-limonene (positive control, 300 mg/kg), or MIBK (1000 mg/kg) and female F344 rats were administered corn oil (vehicle control) or MIBK for 10 consecutive days. Approximately twenty-four hours after the final dose the kidneys were excised and the left kidney prepared and evaluated for histological changes including protein droplet accumulation, immunohistochemical staining for α2u, and proliferating cell nuclear antigen to quantitate renal cell proliferation. The right kidney was prepared for quantitation of total protein and α2u using an ELISA. MIBK elicited an increase in protein droplets, accumulation of α2u, and renal cell proliferation in male, but not female rats, responses characteristic of the α2u-nephropathy MOA. MIBK produced identical histopathological changes in the male rat kidney as compared to d-limonene, an acknowledged inducer of α2u-nephropathy except that the grade of severity tended to be slightly lower with MIBK. MIBK did not induce any effects in female rats. Therefore, renal histopathology, along with the other measures of α2u accumulation, support the

inclusion of MIBK in the category of chemicals exerting renal effects through a protein droplet α2u-nephropathy-mediated MOA. This study was sponsored by the American Chemistry Council Ketones Panel.

466 COMBINATION OF RENAL FUNCTION INVESTIGATION WITH PLETHYSMOGRAPHY IN RATS: REFINEMENT OF THE CORE BATTERY.

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In Safety Pharmacology ICH Guidelines, the investigation of renal adverse effects is not mandatory and is therefore rarely performed during the preclinical phase. On the contrary, plethysmography is now routinely used as part of the Core Battery package to explore respiratory parameters in rodents and specifically designed plethysmographs (Buxco Research Systems) can be used to combine the two types of studies during respiratory investigations. Renal and respiratory functions were assessed after oral administration of theophylline (THEO: 0, 3, 10 and 30 mg/kg) to eight rats. Respiratory parameters were recorded continuously for 6 hours and urine samples were obtained 2, 4 and 6 hours after dosing. At 0, 3, 10 and 30 mg/kg THEO increased the respiratory rate (0, 0, +45 and +79 %, respectively) and minute volume (+9, -2, +51 and +67 %, respectively) without affecting tidal volume. Urine volume was also increased by THEO at all time points, with the largest volumes recorded 2h after dosing (2.5 ± 0.5, 3.3 ± 0.9, 3.6 ± 1.3 and 4.4 ± 1.6 mL, respectively). With the optimized plethysmographs, urine samples were obtained without opening the device, thus inducing minimal impact on the respiratory pattern. This combined study design fulfilled the ICH S7A Guideline requirements for both renal and respiratory functions, and demonstrated both the expected dose and time-related effects. This method associates scientific and ethical refinements and should enable a more extensive evaluation of renal function during preclinical investigations.

467 LINDANE-INDUCED APOPTOSIS IN MDCK CELLS CORRELATES WITH DOWN-REGULATION OF BCL-XL UNDER CONDITIONS OF HYPERTONIC STRESS.

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The cyclodiene pesticide, lindane (LIN), is toxic to the immune, reproductive, and central nervous systems, as well as the liver and kidneys. Free radical generation/oxidative stress has been indicated as a possible mechanism of toxicity in other organ systems, however the mechanism of renal toxicity has yet to be elucidated. Heat/physical stress may alter susceptibility to pesticide toxicity by shifting cellular stress response. Previously, our laboratory has shown a LIN-induced concentration-dependent increase and time-dependent decrease in generation of reactive oxygen species (ROS) and a depletion of cellular glutathione (GSH) levels in MDCK cells. To further evaluate LIN-induced renal toxicity, we measured apoptosis under conditions of hypertonic stress at 2, 6, 12, and 24 hours in MDCK cells treated with vehicle (DMSO), a known oxidant-stress inducing compound, H_2O_2 (1 mM), and LIN (0.01 mM & 0.1 mM). Cells were pretreated with N-acetyl cysteine (NAC) to determine the effect of oxidative stress on cell death. NAC attenuated the apoptotic response for both H_2O_2 and LIN-treated cells under normal conditions. Treatment under conditions of hypertonic stress resulted in an earlier onset of H_2O_2 and LIN-induced apoptosis. Western blot analysis was carried out to determine if LIN exposure under normal and hypertonic stress conditions altered expression of the anti-apoptotic protein, Bcl-xL. LIN down-regulated expression of Bcl-xL at all time points under normal and hypertonic conditions. Control-treated cells showed normal expression of Bcl-xL, with attenuated expression under hypertonic stress. H_2O_2-treated cells showed decreased expression at 6 hours, with a re-expression at 12- and 24- hours for normal treatments, while hypertonic conditions caused an earlier down-regulation of Bcl-xL at 2 hours. Lindane exposure under hypertonic stress caused a time-shift in induction of oxidative stress-induced apoptosis in MDCK cells that correlates to down-regulated expression of the anti-apoptotic protein, Bcl-xL.

468 CHEMICAL-INDUCED POST-TRANSLATIONAL MODIFICATIONS AND THE CONSEQUENT STRUCTURAL AND FUNCTIONAL ALTERATIONS.

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Chemical-induced post-translational modifications (PTMs) can alter the structure of proteins, with consequences that may alter protein function, including interference with protein-protein interactions and subcellular protein localization, and disruption of cell signaling pathways. To identify the impact of PTMs on the structure and function of protein targets in vitro and in vivo, electrophiles with known toxicity were utilized. Hydroquinone, and its thioether metabolites, cause renal proximal tubular cell necrosis and nephrocarcinogenicity in rats. The adverse effects of these chemicals are in part a result of their oxidation to 1,4-benzoquinones (BQ). Cytochrome c (CC) has been studied as a model protein to identify site-specific adductions and the resulting structural and functional consequences. BQ induced PTMs in CC that produce changes in the structure sufficient to inhibit its ability to initiate caspase-3 activation in native lysates, and its ability to promote Apaf-1 oligomerization into an apoptosome complex, in a purely reconstituted system. Also, the impact of PTMs on nuclear actin and chromatin remodeling are under investigation. Actin is a subunit of the INO80 chromatin remodeling complex. Following in vivo BQ dosing in the S. cerevisiae S288C yeast strain, DNA-binding activity of the INO80 complex was decreased, and protein alterations in the INO80 complex was observed by SDS-PAGE/silver staining, suggesting that BQ can alter the structure and function of a chromatin remodeling complex. Ongoing studies are being conducted to determine the exact role PTM-nuclear actin may play in chromatin remodeling. In summary, mass spectroscopic, molecular modeling, and biochemical approaches collectively confirm that electrophile-protein adducts produce structural changes that influence biological function. (GM070890, ES006694, ES007091, ACS-RSG-05-029-01-CCG)

469 TUBEROUS SCLEROSIS-2 AND THE REGULATION OF 8-OXOGUANINE DNA GLYCOSYLASE.

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The Tuberous Sclerosis-2 (*Tsc2*) tumor suppressor gene encodes for the protein tuberin, a multi-functional protein with sequence homology to the GTPase activating protein for Rap1. Mutations in the *Tsc2* gene are associated with the development of renal tumors. The Eker rat contains a germ-line insertion in one of the *Tsc2* alleles ($Tsc2^{Ek/+}$) and when treated with 2,3,5-tris-(glutathion-S-yl)hydroquinone (TGHQ), a redox-active and potent nephrotoxic metabolite of hydroquinone, there is an increase in renal tumors. The constitutive expression of 8-oxoguanine-DNA glycosylase (OGG1) in $Tsc2^{EK/+}$ rats is three-fold lower than in wild-type $Tsc2^{+/+}$ rats, suggesting a novel regulatory role for *Tsc2* in base-excision repair in response to reactive oxygen species induced DNA damage. We investigated the mechanism of OGG1 regulation by *Tsc2*. Tissue lysates along with mRNA from Eker and $Tsc2^{+/+}$ rats treated with TGHQ or saline (control) at 1,2,4,6 and 8 months were taken and probed for OGG1 and *Tsc2* expression. Tuberin and OGG1 protein levels were measurably higher in the $Tsc2^{+/+}$ tissue samples as determined by Western blot analysis. Furthermore, QTRRE cells, TGHQ transformed cell lines from Eker rat renal proximal tubule cells null for *Tsc2*, were transiently transfected with *Tsc2*. Following transfection, tuberin and OGG1 protein levels, determined by Western blot, were significantly higher in transfected versus control cells reaching maximum induction at 24 and 72h, respectively. Moreover, rapid decreases in tuberin expression occurred over 48h with concomitant gradual decreases in OGG1 over 72h. RT-PCR analysis of tissue samples and *Tsc2* transfected cells revealed no changes in OGG1 gene expression indicating, the regulation of OGG1 does not occur at the transcriptional level. On-going studies are being conducted to identify additional factors that may play a role in the observed OGG1 protein expression. (GM039338, GM070890, ES006694)

470 MALDI TISSUE IMAGING OF RENAL TUMORS THE IN EKER RAT.

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Eker rats, carrying a germline mutation in the Tsc-2 gene (Tsc-2 Ek/+) were treated with a renal carcinogen 2,3,5-tris-(glutathion-S-yl)hydroquinone (TGHQ), in order to determine protein responses to a tumorigenic environmental toxicant in a genetically susceptible animal. Renal tumors in the Tsc-2Ek/+ rat result from loss of heterozygosity of the normal allele of the Tsc-2 gene, observed in preneoplastic lesions and tumors. MALDI tissue imaging was used to compare protein signature in normal, injured, and tumor bearing kidneys from saline and TGHQ treated Tsc-2+/+ (wild type) and Tsc-2Ek/+ rats. MALDI tissue images are three-dimensional arrays of data from tissue sections in which each pixel on the XY plane of the tissue section contains a MALDI mass spectrum. Images of the tissue sections are created based on the masses of proteins therein. Our results showed that a 9955 Da protein was up-regulated in tumors when compared to "adjacent normal' tissue. In contrast, a 14212 Da protein was found to be more abundant in normal tissue than in tumors. Both 9955 and 14212 Da proteins were found in the perchloric acid soluble fraction and identified by LC MS/MS as acyl CoA binding protein (ACBP) and ribonuclease UK114, respectively. UK114 and ACBP have functions in common. MALDI mass spectrum profiling is presented as a tool to guide generation of hypotheses as to which of many possible functions are being performed by UK114

and ACBP in the normal, injured, and neoplastic rat kidney. UK114 down-regulation may potentiate renal cell carcinoma by loss of its ribonuclease activity. Interestingly recent literature suggests that the ACBP gene is transcribed in response to activation of peroxisome proliferator-activated receptor (PPARγ). The role that UK114 and ACBP may play in renal tumor development is under investigation. The utility of MALDI tissue imaging profiling to refine hypotheses in signaling cascades through the Tsc-2 pathway, leading to changes in protein expression, is explored. (GM039338, ES006694)

471 STRAIN DIFFERENCE OF URINARY OXALATE AND ELECTROLYTES IN ETHYLENE GLYCOL TREATED WISTAR AND F344 RATS.

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Ethylene glycol (EG), the main component of antifreeze, causes metabolic acidosis, acute renal failure, and rapid cerebral edema upon ingestion. Although EG itself is relatively nontoxic, it is converted to toxic metabolites such as oxalate which accumulates in the kidney as calcium oxalate monohydrate (COM). Previous studies show that the Wistar rat strain is more sensitive to COM accumulation and hence to the renal toxicity of EG than is the F344 strain. Supersaturation of calcium oxalate in tubular fluid contributes to the amount of calcium oxalate monohydrate (COM) crystal formation in the kidney. This study was designed to compare the concentrations of several electrolytes and test the hypothesis that the urinary supersaturation of calcium oxalate of a Wistar rat is lower than that of an F344 rat, thereby allowing for greater COM formation/accumulation in the Wistar rat. Male Wistar and F344 rats were treated with EG (0.75%) in drinking water for 8 weeks. Rats were placed in metabolic cages and urine was collected for 24 h after 2,4,6 and 8 weeks. Urine was analyzed for key electrolytes and the calcium oxalate supersaturation was calculated. Prior to EG treatment, there were no differences in urinary oxalate, calcium and creatinine levels, nor in oxalate supersaturation. After treatment, urine oxalate excretion was markedly greater in the Wistar rats than in the F344 rats, while urine calcium was lower in Wistars. These studies suggest that urine electrolyte changes and oxalate supersaturation occur to a greater extent in the Wistar rat, in agreement with its greater sensitivity to EG toxicity. Supported in part by research agreement with American Chemistry Council.

472 A METABONOMIC INVESTIGATION INTO THE DIURETIC EFFECTS OF BROMOETHANAMINE.

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COMET 2 aims to use metabolism driven 'top-down' systems biology as a tool to direct research to ascertain the mechanisms of toxicity for selected compounds. Bromoethanamine (BEA) is a potent nephrotoxin, inducing renal papillary necrosis following a single dose. Although it has been extensively studied in experimental animals the mechanisms by which BEA induces toxicity are poorly understood, but increased urinary output, increased urinary glutaric and adipic acid and decreased urinary dimethylglycine and methylamine concentrations have all been detected. To enable non-BEA specific metabolic changes to be eliminated a forced diuresis and volume overload study was undertaken. Furosemide (FS) is a highly potent loop diuretic, exerting its action by inhibiting Na+, K+ and Cl- reabsorbtion in the thick ascending loop of Henle. Male Sprague-Dawley rats were given a single i.p. dose of either 50, 150, or 250 mg/kg BEA or a single oral dose of either 25 ml/kg saline or 25 ml/kg saline and 30 mg/kg FS. Urine was collected 24hrs prior to dosing, 0-4, 4-8 and 8-24 hrs post dosing. In all dose groups a marked polyuria was detected with metabonomic data generated using high-field solution state NMR and UPLC-MS analysis. 1H NMR urinary spectral profiles were modelled using O-PLS-DA to determine the metabolic differences between animals with forced diuresis and those administered BEA. UPLC-MS data were analysed using MarkerLynx to identify differences between the dose groups. Metabonomic analysis of the urinary NMR and UPLC-MS profiles enabled the identification of non-specific metabolic changes associated with polyuria, including decreased dimethylglycine and methylamine, thus facilitating the identification of metabolic changes directly associated with BEA.

473 IDENTIFICATION OF PROBABLE GENOMIC AND URINARY PROTEIN NEPHROTOXICITY BIOMARKERS IN MICE.

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Nephrotoxicity is a recurring concern in drug development. Since the existing nephrotoxicity biomarkers such as serum creatinine and blood urea nitrogen are not sensitive enough to indicate early renal injury, many research efforts have focused on finding more robust nephrotoxicity biomarkers. Although much data has accumulated for potential nephrotoxicity biomarkers in rat, relatively less is known in mice. To address this, mice were treated with a single dose of a nephrotoxicant (Schering-Plough Compound X) and tissues were harvested at different time points up to 24 hours. RNA was extracted from kidneys and subjected to gene expression analysis using the Affymetrix GeneChip platform. Subsequent RT-PCR analysis based on leads generated from microarray identified a set of genes whose expression changes correlated very well with histopathological findings in individual animals. Furthermore, expression changes of some of these genes preceded the onset of tissue damage and may serve as predictors of renal toxicity in mice. Pre-dose and post-dose urine collected at the 24 hour time point was subjected to urinary protein analysis using ELISA methods. The results from individual mice demonstrated that the urinary levels of proteins such as Lipocalin 2 and Osteopontin correlated well with histopathological findings and might be good indicators of nephrotoxicity in mice.

474 WATER ARSENIC AND DAIRY FOOD SAFETY.

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In 2001, the US EPA set a new Maximum Contaminant Level (MCL) for arsenic (As) in drinking water at 10 ppb. The change in the arsenic MCL raised concerns for dairy producers whose livestock drink water that exceeds 10 ppb MCL. This study was designed to determine if food products derived from cattle drinking water containing arsenic concentrations greater than 10 ppb contained arsenic. Dairy herds were selected based on locations known to have elevated arsenic levels in ground water. Herds were separated into groups based on the concentration of arsenic (ppb) in well water. Samples of all feeds were collected and analyzed to account for any arsenic the cows may have ingested through diet. Arsenic elimination was determined by measuring creatinine corrected urine arsenic concentration. Bulk tank milk samples, cheese made from milk from these herds and tissues from cull cows were analyzed for the presence of arsenic using ICP-MS at a 5ppb limit of quantitation. Milk, whey, cheese, liver, pancrease, and skeletal muscle samples were below the limit of detection for arsenic. Urine arsenic concentration correlated with water arsenic intake. This study did not identify human risk from dairy products derived from dairy cattle drinking water containing up to 114 ppb arsenic.

475 FISH CONSUMPTION ADVISORIES: TOXICOLOGICAL RISK AND NUTRITIONAL BENEFIT MESSAGES TO SENSITIVE POPULATIONS.

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The concept of combining both toxicological risk and nutritional benefit information into fish consumption advisories is gaining appeal. These efforts are particularly relevant when formulating advice for sensitive populations. Fish consumption advisories are issued by 48 states to warn the public of possible toxicological threats from consuming certain fish species. Approximately 88% of advisories are due to just a handful of contaminants: mercury, polychlorinated biphenyls (PCBs), chlordane, dioxins, and dichloro-diphenyl-trichloroethane. We conducted a comparative analysis of health messages contained in advisory Web sites issued by states that sensitive groups can access through the Environmental Protection Agency's National Listing of Fish Advisories. All advisories targeted children, 90% (43) targeted pregnant women, and 58% (28) targeted women of childbearing age. Twenty-six chemical contaminants were responsible for advisories issued by states. All advisories based advice on mercury risks and 75% (36) based advice on PCB risks. Only 6 advisories addressed single contaminants, while the remainder, 42, based advice on 2 to 12 contaminants. Results also revealed that advisories associated a dozen contaminants with specific adverse health effects. For example, risk of nervous system damage was associated primarily with mercury, but also with 7 other contaminants. 23% of advisories did not address the nutritional value of fish. These findings highlight the complexity of multiple contaminant exposure and implications for human health, as well as a lack of communication regarding the multiple potential health benefits conferred by specific fish nutrients. This work was supported by NIEHS 09601, 12762, 7033; NSF 434087; and EPA R 826886.

476 NATURAL IS NOT ALWAYS SAFE: A LESSON FROM THE LITERATURE ON THE USE OF HERBAL PRODUCTS.

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The use of herbal products has seen a tremendous increase over the past several years; their popularity is linked to the belief that, as they are natural, they are devoid of possible adverse side effects often associated with pharmaceutical drugs.

Notwithstanding the known adverse effects of botanicals such as Kawa (known to be associated with hepatotoxicity) and of Ephedra (removed from the market because of its cardiovascular and psychiatric adverse effects), even some of the most widely used botanicals, such as Hypericum perforatum (St. John's wort), Ginkgo biloba (Ginkgo), and Panax ginseng (Asian ginseng) are not devoid of potential undesired side effects. Hypericum extracts, used to treat mild-moderate depression, can induce the cytochrome-P450 system thus reducing the efficacy of several drugs (e.g. cyclosporines, oral contraceptives, warfarin). Reported adverse effects include photosensitization, psychosis, sedation, decreased libido, and convulsions. Ginkgo leaves extracts, used for cognitive disorders and peripheral vascular disease, can cause bleeding (persistent postoperative bleeding, ocular hemorragies). Moreover, contact dermatitis, morbilliform eruptions, seizures, and ventricular arrhythmias have been reported, in addition to interactions with warfarin, trazodone, and anticonvulsants. Ginseng root extracts, used as "adaptogen" remedy (that should protect against stress), can affect the cardiovascular system by interfering with platelet aggregation, and causing changes in blood pressure. Ginseng may also produce manic symptoms, especially in patients under antidepressant medications. In addition to adverse health effects, some herbal products may also be contaminated with aflatoxins, pesticides or heavy metals. Given that herbal products are consumed by millions of individuals, and that their commercialization is not subject to the same safety assessment as pharmaceutical drugs or other chemicals, there is a need for toxicologists to be fully aware of the potential adverse health effects, contaminations and interactions linked to the use of such products.

477 IDENTIFICATION AND CHARACTERIZATION OF TOXICITY OF CONTAMINANTS IN PET FOOD LEADING TO AN OUTBREAK OF RENAL TOXICITY.

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An outbreak of renal toxicity in cats and dogs was traced to consumption of food from a contract manufacturer, specifically to a supply of wheat gluten that was an ingredient. Screening by HPLC-mass spectrometry detected a significant concentration of melamine (M) in the wheat gluten, along with lower levels of related compounds, including ammeline (An), ammelide (Ad), ureidomelamine and methylmelamine. An and Ad are deamination products of the bacterial metabolism of melamine; therefore, we postulated that a third product of deamination, cyanuric acid (CA), must also be present. More extensive analytical investigation revealed the presence of CA and a concentration of M much higher than had been measured previously. Both melamine and cyanuric acid had been extensively tested for toxicity and neither produce acute renal toxicity. We tested the potential of all identified contaminants to produce cytotoxicity in feline and canine kidney cells; none were active at relevant concentrations. The clinical findings in cats and dogs included crystals in renal tubules. We hypothesized that one or a combination of the contaminants produced toxicity via formation of crystals leading to renal failure by obstruction. We evaluated in a rodent model the potential of An or Ad alone (at 100 mkd) to produce crystals, because these were the two contaminants with the lowest solubility; and of two mixtures, M/CA (400/400 mkd), and M/Am/Ad/CA (400/40/40/40 mkd). Neither An nor Ad produced renal precipitates or renal toxicity. Both mixtures produced significant levels of crystals in kidney tubules and substantial renal toxicity. The chemical identity of the crystals was confirmed by IR microspectroscopy as being mainly melamine cyanurate, a complex that is virtually insoluble. We believe that the compounds were tightly associated in the contaminated gluten, dissociated in the acidic lumen of the stomach, were absorbed at different rates because of differing pKa then recrystallized in kidney tubules.

478 DISPOSITION OF MELAMINE IN PIGS.

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Melamine-contaminated pet food was recently added as a supplement to livestock feed. There is little or no information concerning the pharmacokinetics of melamine in livestock, and the aim of this study was to obtain pharmacokinetic parameters for this contaminant in pigs. Melamine was administered intravenously to five weanling pigs at a dose of 6.13 mg/kg and plasma samples were collected over 24 hours, extracted for melamine, and then analyzed by HPLC-UV. The data was shown to best fit a one-compartment model with melamine's half-life of 4.04 (± 0.37) hours, clearance of 0.11 (± 0.01) L/hr/kg, and volume of distribution of 0.61 (± 0.04) L/kg. These data are comparable to the only mammalian study in rats and suggests that melamine is readily cleared by the kidney and there is unlikely to be significant tissue binding. Further tissue residue studies are required to assess the depletion kinetics of this contaminant in the pig which will determine whether residue levels in the kidney should be of public health concern if pigs were exposed to a similar dose. (Supported by USDA-CSREES 2006-45051-03471)

479 ACRYLAMIDE: TOXICOKINETICS IN HUMANS.

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The rodent carcinogen acrylamide (AA) is formed during the preparation of starch-containing foods. AA is metabolized to glycidamide (GA) by CYP 2E1. Both AA and GA are conjugated with glutathione. After enzymatic processing, N-acetyl-S-(2-carbamoylethyl)-L-cysteine (AAMA) and the regioisomers rac-N-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine and rac-N-acetyl-S-(1-carbamoylethyl-2-hydroxy-ethyl)-L-cysteine (GAMA) are excreted with urine. In humans, AAMA can be sulfoxidized to AAMA-sulfoxide, which is also excreted in urine. GA as genotoxic agent may be responsible for initiation of tumors by AA. This study determined the toxicokinetics of AA in humans after oral administration in doses similar to daily dietary exposure to assess potential species-differences in AA-bioactivation. The study was performed with informed consent and the study protocol was approved by the local institutional review board. Two doses of 13C3-AA (0.5 and 20 µg/kg b.w.) were administered orally to male and female human subjects (n = 3/gender) in 100 ml of water. After administration, urine samples were collected for 94 hours in intervals. The urinary concentrations of 13C3-AAMA, 13C3-GAMA and 13C3-AAMA-SO were monitored by hydrophilic interaction liquid chromatography-tandem mass spectrometry (HILIC-MS/MS). The overall recovery in the low dose study was 71.26 ± 5.09 % of dose (0-46 hours) with 51.74 ± 3.78 % recovered as 13C3-AAMA, 6.32 ± 0.88 % as 13C3-GAMA and 13.20 ± 3.17 % as 13C3-AAMA-SO. Half lives for 13C3-AAMA, 13C3-GAMA and 13C3-AAMA-SO were 11.60 ± 2.37, 19.78 ± 5.99 and 28.23 ± 12.25 hours. The total recovery in the high dose study was 70.03 ± 3.12 % of the administered dose (0-94 hours) with13C3-AAMA, 13C3-GAMA and 13C3-AAMA-SO accounting for 49.16 ± 2.84, 6.41 ± 1.21 and 14.46 ± 3.01 %. Half lives for 13C3-AAMA, 13C3-GAMA and 13C3-AAMA-SO were 14.16 ± 1.13, 26.29 ± 2.49 and 21.48 ± 4.64 hours, respectively. The obtained results suggest that the extent of AA bioactivation to GA in humans is lower than in rodents.
Supported by the Deutsche Forschungsgemeinschaft.

480 A 12-WEEK TOXICOLOGICAL STUDY OF ORALLY ADMINISTERED ACRYLAMIDE IN JUVENILE RATS.

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Acrylamide (AA) has been reported to be formed in fried and baked foods with various concentrations. Human exposure levels to AA from cooked foods in childhood are estimated to be higher than those in adults. Although numerous toxicological studies in adult rats have shown that the nervous system and testes are principal sites of the toxicities of AA, juvenile toxicological data are extremely limited. In the present study, pathological examinations were performed in rats administered AA after birth for 12 weeks. A total of 12 dams of F344 rats were divided into 4 groups and given AA in the drinking water at 0, 10, 20 and 40 ppm during lactation period. The weaned offspring were also given AA at the same dose levels until postnatal week 12. As results, actual AA intakes at 10, 20 and 40 ppm groups after weaning were equivalent to 1.0, 2.1 and 4.4 mg/kg/day body weight, respectively, in males and 1.2, 2.5 and 4.9 mg/kg/day body weight, respectively, in females. Treatment with AA caused a slight decrease in body weight in 20 and 40 ppm females and a slight increase in relative thyroid and spleen weights in 40 ppm females. Histopathologically, AA-related findings were observed in testes, epididymides and heart. In the testes and epididimides, slight seminiferous tubule atrophy and desquamated epithelium in ducts, respectively, were observed at 40 ppm. In the heart, the incidence and degree of myocarditis especially localized in basis cordis were significantly increased in 40 ppm males. No significant findings were recorded in other organs/tissues including brain, sciatic nerve, thyroids and spleen. In comparison with previous reports, the testicular toxicity-inducible dose levels were similar to the present study. Although a further study with scaled-up protocol is now performed to re-confirm, if the cardiac change is induced by a AA-specific reaction, it might be speculated that myocyte proliferation in the postnatal period is at least partly associated with the induction of such lesion.

481 DIET BASED EXPOSURE TO ACRYLAMIDE IN FINLAND.

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It is now recognized that acrylamide is a concern not only in potatoes or specific starchy foods, but is a much broader food issue. Use of the terms high and low to describe levels of acrylamide in a food is ambigious and misleading - there is no basis for the use of these comparative terms except to describe the relative range of values encountered. What is required is dietary exposure data - generated with validated methods - from foods of many countries to assess the range of potential intake.

We analyzed three hundred and one samples with a completely validated HPLC/MS/MS method (the relative standard deviations for repeatability and day-to-day variation were below 15% in all food matrices, particiaption to two intercalibaration trials)which included more than 30 different types of food and foods manufactured and prepared in various ways. Samples were ramdomly selected from local grocery stores in Helsinki, Finland during 2002, 2004 and 2006 and thus they do not necessarily reflect the national food supply. Acrylamide was detected at levels, ranging from nondetectable to 1480 µg/kg level in solid food, with crisp bread exhibiting the highest levels. In drinks, the highest value (29 µg/L) was found in regular coffee drinks. Acrylamide concentration in some cases refers only to a single randomly selected sample of each specific product. The data are a general quide to acrylamide concentrations in selected segments of the Finnish food suply. The levels of acrylamide in these particular foodstuffs from the Finnish market were at the same level as those reported from the other countries. In the view of its daily consumption, bread may represent one of the most important contributors to dietary exposure of acrylamide. Many of the products which contained moderately high levels of acrylamide are popular snaks among young people. To decrease the overall exposure to these side products of food processing, attemps should be made to devise improved food making processes, perhaps using new raw materials, to prevent the formation of food-borne hazards.

482 RELATIVE AMOUNTS OF THE MYCOTOXIN DEOXYNIVALENOL IN FOODS PREPARED FROM WHEAT FLOUR UNDER COMMERCIALLY RELEVANT CONDITIONS.

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Deoxynivalenol (DON) is a *Fusarium* mycotoxin found in wheat, barley, corn and in food products of cereal origin. DON causes variable effects in animals including feed refusal, decreased weight gain, or altered immune function. DON's human health effects are not well understood, however, there is concern that it might adversely affect growth or immune function in children. Some cooking methods reduce DON levels in foods while others do not. DON concentrations in wheat flour and flour-based products made under commercially relevant conditions were compared by gas chromatography (GC) - electron capture detection to assess DON reduction. Mass balance was estimated from DON concentration and product yield data. The concentrations of DON detected in cookies, crackers, and pretzels ranged from 67% (cookies) to 113% (pretzels) of the amount in flour (<0.5 ppm). Greater reductions, approximately 70%, were found in bread. Mass balance calculations yielded a similar pattern: total DON in the batches of cookies, crackers, and pretzels ranged from 93% to 110% of the amount in flour whereas 45% was found in bread. This indicates that reduced DON concentrations in bread partially resulted from dilution effects related to its relatively high yield (1:1.6, flour:bread). DON concentrations (detection by GC-mass spectrometry) of fried donuts were reduced about 75% and, like bread, dilution by other ingredients contributed to reduction. These studies constitute the first phase of investigations on the fate of DON in foods prepared under commercial conditions and are therefore preliminary. They nonetheless show that significant amounts of DON survived processing and were found in the cookies, crackers, and pretzels. A modest reduction occurred in baked bread and reductions were also found in fried donuts.

483 IN VITRO CHARACTERIZATION OF MYCOTOXIN BINDING AGENTS INCLUDED IN ANIMAL FEEDS IN MEXICO.

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This study was conducted to characterize and compare 12 different products distributed in Mexico as mycotoxin binders utilizing: 1) equilibrium isothermal analysis for Aflatoxin B1 (AfB_1) adsorption and 2) mineralogical probes. The binders included Volclay (Volclay), Zeotek, Duotek (Nutek), Mexsil (Karluis), Mycosil (Petrotec), Mycosorb (Alltech), Klinsil (Helm), Toxinor (Norel), Micofix (Biomin), Fixat (Sud-Chemie), Myco-Ad (Avimex) and Milbond (Milwhite) and were compared to NovaSil (Engelhard). Isotherms for AfB_1 (Sigma Chemical) adsorption were conducted at pH 2 and pH 6.5 in triplicates (5 ml per tube) that consisted of 11 different concentrations of AfB_1 with 0.1 mg of each binder. Controls consisted of water, water plus binder and AfB_1 (8 ppm). Mineralogical analysis included determination of swelling volume (SV) to ascertain presence of smectites; and fractionation procedures to calculate the percentages of sand, silt, and clay in each binder. The most effective binders were used for single-concentration isothermal analyses with AfB_1 (8 ppm) utilizing sand and silt fractions, as well as the parent product. Computer-generated isotherm data were fitted using the Langmuir model, and semi-empirical parameters of Q_{max} and K_d were estimated. L-shaped curve characteristics were observed for the binders in the isothermal sorption of AfB_1 at pH 6.5 with varied r^2 values. The most effective binders for AfB_1 at both pH conditions were NovaSil (Q_{max} = 0.40), Milbond, Myco-Ad and Volclay while the least effective was Mycosorb (Q_{max} = 0.01). In the single-concentration adsorption analyses, NovaSil exhibited the highest percentages of AfB_1 binding in all the fractions. In general, NovaSil demonstrated the highest sorption capacity and affinity for AfB_1. (Supported by the graduate fellowship PROMEP/103.5/05/1673 from Mexico).

484 EVIDENCE FOR FUMONISIN-CORN MATRIX BINDING DURING NIXTAMALIZATION.

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Fumonisins are mycotoxins produced by *Fusarium verticilliodes*. They are found in corn and corn-based foods. Nixtamalization (cooking in alkaline water) reduces detectable fumonisins; however, their concentrations in foods can be underestimated if fumonisin-matrix binding occurs. The purpose of this study was to determine if fumonisin FB_1 (FB_1) binds to the corn matrix during nixtamalization. A modification of the method of Park et al. (*Food Addit. Contam.* 21: 1168-78, 2004) was used in which (a) free fumonisins were quantified by HPLC; (b) protein bound fumonisins (FB_1 plus hydrolyzed FB_1 (HFB_1)) were then extracted with sodium dodecyl sulfate (SDS) and quantified as HFB_1 following base hydrolysis of the extract; and (c) the remaining (residual) bound fumonisins were quantified as HFB_1 after the SDS-extracted residue was base hydrolyzed. Whole kernel corn (23 $\pm$ 11.5 ppm = 32.3 $\pm$ 15.9 nmol/g FB_1; 0.33 $\pm$ 0.10 ppm = 0.81 $\pm$ 0.25 nmol/g HFB_1) and nixtamalized and sham nixtamalized samples (base omitted) of the corn were evaluated. About five fold more (nmol/g) protein bound fumonisin was detected in the nixtamalized samples (0.40 $\pm$ 0.20 ppm detected as HFB_1 = 0.98 $\pm$ 0.49 nmol/g) than in the uncooked (0.17 $\pm$ 0.12 nmol/g) or sham nixtamalized (0.17 $\pm$ 0.05 nmol/g) corn. Residual bound fumonisins in the nixtamalized samples (0.32 $\pm$ 0.17 ppm detected as HFB_1 = 0.79 $\pm$ 0.42 nmol/g) were about 10 to 15 times more than in the uncooked (0.05 $\pm$ 0.01 nmol/g) or sham nixtamalized (0.07 $\pm$ 0.01 nmol/g) corn. This indicates that fumonisins bound to the matrix during nixtamalization, but that the amount of binding was low: protein bound plus residual bound fumonisins detected were only about 5 to 10% of the amounts of free FB_1 plus HFB_1 in the uncooked, nixtamalized (3.31 $\pm$ 1.08 ppm = 4.59 $\pm$ 1.50 nmol/g FB_1; 4.79 $\pm$ 0.47 ppm = 11.8 $\pm$ 1.16 nmol/g HFB_1), and sham nixtamalized (11.0 $\pm$ 3.22 ppm = 15.3 $\pm$ 4.47 nmol/g FB_1; 0.13 $\pm$ 0.16 ppm = 0.32 $\pm$ 0.39 nmol/g HFB_1) corn.

485 COMPARATIVE AND COMBINATIVE CYTOTOXIC EFFECTS OF AFLATOXIN B1 AND FUMONISIN B1 ON HUMAN ESOPHAGEAL CELLS.

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Aflatoxin B1 (AFB1) is a risk factor of liver cancer. Fumonisin B1 (FB1) is a carcinogen and a promoter in animals and was categorized as possibly carcinogen to humans (Group 2B) by IARC. Associations between dietary FB1 exposure and esophageal cancer were suggested by studies in South Africa and China. Co-contamination of AFB1 and FB1 is found in human food. The combinative toxic effects of co-exposure to these two mycotoxins are of public concerns. In this study, comparative and combinative cytotoxic effects of AFB1 and FB1 were tested in normal human esophageal epithelial cells, Het-1A, and esophageal carcinoma cells, KYSE 150, by PreMix WST-1 cell proliferation assay. Cells were seeded in normal media at 96 wells for 24 h and were replaced with media containing 200, 100, 10, 1, and 0.1 µM FB1 or 100, 10, 1, 0.1, and 0.01 µM AFB1. The time-course cytotoxic effects of FB1 and AFB1 on the growth of these cells were evaluated after 24, 48, 72, and 96 h, respectively. No significant growth inhibition was found on Het-1A cells treated with AFB1 for 24 and 48 h. However, significantly inhibitory effect was found in cells treated with AFB1 at higher than 1 µM for 72 and 96 h and the maximum inhibition rate of 55.88% was observed in 100µM treated for both 72 and 96 h. Treatment of AFB1 significantly inhibits the growth of KYSE 150 cells. The inhibition rate reached 59.18% at 100µM for 24 h. The IC50 is 30.55, 24.11, 8.11, and 8.34µM for 24, 48, 72, and 96 h treatment, respectively. Treatment of FB1 for 24 or 48 h was no significantly cytotoxic effect on the Het-1A cells and the inhibition rates of 22.9% and 33.83% were observed in cells treated with 100 and 200 µM FB1 for 96 h. On the other hand, growth inhibition was found in KYSE

150 cells treated with higher concentrations of FB1 for 24, 48, 72, and 96 h, with the maximum inhibition rate of 50.84% observed at 200µM for 96 h. These results showed that KYSE 150 cells are more sensitive than the Het-1A in AFB1- or FB1-induced cytotoxic effects. Combinative cytotoxicity tests showed additive interactions of AFB1 and FB1 in these two cell lines.

486 DEVELOPMENT OF ADSORPTIVE MATERIALS FOR THE REMEDIATION OF FUMONISIN B1 CONTAMINATED FOODSTUFFS.

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Fumonisin B1 (FB1) has been implicated in the etiopathogenesis of disease in animals and humans. In this study, the primary objective was to identify an adsorbent material that would sequester FB1 from aqueous solutions both in vitro and in vivo. Ferrihydrite (FH) and Wyoming Sodium Montmorillonite (SWY-2) were added at 6 concentrations (0.25mg-5mg) to samples that contained 4ppm FB1 at pH7 and pH2 and allowed to equilibrate before o-pthaladehyde derivitization and HPLC analysis. Results indicated that SWY-2 showed significant binding (95-99%) at concentrations equal to or higher than 0.5 mg at pH7 but very little affinity at pH 2. FH showed significant binding (92-99%) at pH 7 and pH 2 at sorbent concentrations above 1.0 mg. Equilibrium isothermal analysis for FB1 sorption was conducted at 4°, 25°, and 37°C to ascertain the binding capacity of FH and SWY-2 and to estimate the thermodynamics of the binding reaction. All isotherm data were fit to the Langmuir equation to determine adsorption capacity (Q_{max}). Results indicated that Q_{max} values were equal to 0.023 and 0.126 mol FB1/ kg clay for FH and SWY-2, respectively. The minimal effective concentration (MEC) for FB1 toxicity in the hydra assay was derived from logarithmic dosimetry analysis (LDA) and was equal to 300ppm. A concentration higher than the established MEC (400 ppm FB1) was added to the hydra medium and served as a positive control toxicity indicator for FB1. In the bioassay, 2.4% FH and 0.35% SWY-2 were added based on Q_{max} values for FH and SWY-2. Results confirmed that FB1 at 400 ppm was lethal to hydra within 48hrs and both FH and SWY-2 prevented the lethal toxic endpoint in the hydra bioassay. No toxicity was observed for FH or SWY-2. Studies are ongoing to optimize these and other multifunctional materials for the remediation of mycotoxin contaminated foods (Supported by USAID LAG-G-00-96-90013-00 and NIEHS P42 ES04917).

487 ACCUMULATION OF SPHINGOID BASES, THE STRUCTURAL SPHINGOID BASE ANALOG FTY720 AND THEIR PHOSPHORYLATED METABOLITES IN BLOOD, PLASMA AND DECIDUA OF SWV AND LMBC MICE TREATED WITH FUMONISIN B1 OR FTY720.

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Fumonisins (FB) are mycotoxins in maize and inhibitors of ceramide synthase (CS). FB1 has been shown to cause neural tube defects (NTD) in mice with the LMBc strain being more sensitive than the SWV or other strains. FTY720 (FTY) is a sphingoid base analog derivative of myriocin, another fungal metabolite. FTY is currently in human clinical trials as a prototype immunosuppressant drug, and our results show that it also causes NTD in mice. Phosphorylated sphingoid bases and phosphorylated FTY (FTY 1-P) are ligands for the lysophospholipid receptors known as S1P receptors (S1P 1-5). We have shown that sphinganine (Sa) and Sa 1-phosphate (Sa 1-P) are significantly elevated in fetal liver of LMBc mice consuming FB in the diet. The role of elevated S1P receptor agonists in NTD development is however unknown. The present study was undertaken to see if Sa 1-P and FTY 1-P were elevated in blood, plasma and other tissues of SWV and LMBc mice treated with FB (ip injection) or FTY (gavage). The results show that in FB treated mice the levels of sphingoid bases and sphingoid base 1-phosphates are significantly elevated in blood, plasma and decidua. In the blood and plasma of FB treated mice the amount of Sa and Sa-1P was significantly greater in the LMBc mice as was the NTD incidence compared to the SWV mice. In the FTY treated animals FTY 1-P was significantly elevated in plasma and decidua in both strains although the levels in LMBc mice were greater than in the SWV as was the incidence of NTD. Similarly, following FB treatment the incidence of NTD after exposure to FTY was greater in the LMBc mice. The results suggest that elevation in S1P receptor agonists may play a mechanistic role in NTD development in mice.

488 ESTIMATION OF OCHRATOXIN A IN TURKISH POPULATION: AN ANALYSIS IN URINE AS A SIMPLE, SENSITIVE AND RELIABLE BIOMARKER.

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Ochratoxin A (OTA), a mycotoxin produced by several fungi of Aspergillus and Penicillium species in the food chain, has nephrotoxic, hepatotoxic, carcinogenic, teratogenic and immunotoxic effects. OTA is classified as a possible carcinogen to human (Group B) by IARC. Exposure to this mycotoxin and evaluating its presence in populations has become highly important.

In this study, the validation of a simple, sensitive and accurate analytical method for the urinary monitoring OTA levels as a marker for OTA human exposure is presented. It has been preferred for being less invasive than blood monitoring. This method involves extraction with 5% NaHCO3, commercial immunoaffinity column for clean-up and reversed phase high performance liquid chromatography with fluorescence detector (HPLC-FD). The method was found to be selective for OTA in matrices tested. Recovery rates from human urine samples ranged from 85.5 to 110.5 %. The linearity of the spiked samples was 0.9922 for OTA levels of 0.02, 0.05, 0.1, 0.5 and 1.0 ng/mL in urine samples. The precision (RSDr) was between 3,9 and 8,86 %. The limit of detection (LOD) was found to be 0.006 ng/mL while the limit of quantification (LOQ) was 0.018 ng/mL.

On the other hand, the aim of this reseach was to evaluate population contamination of OTA, therefore we follow up OTA levels in 235 urine samples of inhabitants from different cities in Turkey and the presence of OTA was found in 196 samples, at concentrations above the LOD, ranged between 0.006 and 0.075ng/mL.

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489 MONOCLONAL ANTIBODIES AGAINST OTB: POTENTIAL FUTURE APPLICATIONS.

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Ochratoxins, common contaminants of food and animal feed, have been demonstrated to be toxic and carcinogenic in animals and to be associated with the occurrence of human renal disease. They are therefore of great interest due to their potential impact on human health and economics. Ochratoxins comprise a mycotoxin family of ochratoxin A (OTA), the most ubiquitous and presumably the most toxic member, ochratoxin B (OTB), not as frequently detected and therefore not as intensively studied as OTA, but with seemingly a different mode-of-action than OTA, and the rarely detected ochratoxin C (OTC). In order to causally relate observed in vivo toxicities with the different ochratoxins a monoclonal antibody against OTB (OTB-mAb) allowing the distinction of OTA and OTB was generated (Heussner et al., FCT, 2007). Subsequently, this OTB-mAb has been purified and characterized with particular regard to its application in ELISA and Western analyses. OTB-mAb has been purified from hybridoma supernatant (clone 2F1.E10) via protein G sepharose affinity chromatography and the identity (IgG1 with kappa chain) confirmed. Cross-reactivity with structure related molecules (i.e. OTA, coumarin, phenylalanine) was tested using an indirect, competitive OTB-ELISA. Moreover the OTB-mAb demonstrated specificity in various Western analyses (SDS-PAGE, immunoblotting and dot blotting). Application-dependent detection of OTB via OTB-mAb is possible in a broad concentration range (pM to µM) and with different sensitivities (Western analysis > ELISA), thus confirming the value of this OTB-mAb as a specific and sensitive tool for mycotoxin research.

490 NOVASIL CLAY INTERVENTION IN GHANAIANS AT HIGH RISK FOR AFLATOXICOSIS: IMMUNE PARAMETERS AS BIOMARKERS OF EFFECT.

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Immunotoxicity of aflatoxins (AFs) has been reported in animals and humans and may play an important role in aflatoxicosis and infectious disease rates in the developing world. To further elaborate immune parameters as biomarkers of effect for AF exposure, a total of 17 cellular immune parameters involving T-cell, B-cell and natural killer (NK) cell functions were applied to the efficacy evaluation of a 3-month intervention trial with NovaSil clay (NS) in a Ghanaian population at high-risk for aflatoxicosis. Immune parameters were measured in peripheral leukocytes by flow cytometry and serum AFB1-albumin adducts were measured by radioim-

munoassay. At the baseline prior to intervention, the study subjects with high AF exposure (>median level of AFB-albumin adduct) had a lower percentage of positive CD3-CD56+CD16+ cells (median = 16.17%) than those with low AF exposure (22.12%, p<0.01). The subjects with high AF exposure had a higher percentage of positive perforin expressing CD3-CD56+ cells (median = 38.42%) than those with low AF exposure (30.07%, p<0.05). After the 3-month intervention with NS (3 g/day), the NS group had a significantly higher percentage (median = 20.85%) of positive CD8+CD45RA+CD27- cells compared to subjects in the placebo group (13.48%, p<0.01). A significant dose*time interaction was found for this parameter (p = 0.001). Correlation analysis over the 3-month study period indicated a difference in trends of AF exposure with the percentage of positive CD3-CD56+ NK cells in the NS group compared to the placebo group (p = 0.008). These data indicated that high AF exposure significantly impaired function of subsets of NK cells and that NS intervention could restore the NK cell-mediated immune function through reducing AF exposure. (Supported by USAID LAG-G-00-96-90013-00).

491 SAFETY EVALUATION OF CARRAGEENAN FOR USE IN INFANT FORMULA.

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Carrageenan, a food ingredient manufactured from red seaweed, is permitted for use in numerous food applications in the USA under 21CFR 172.620 based on Good Manufacturing Practices; in Europe, under Council Directive 95/2/EC, Annex 1 – E 407 at *quantum satis* (with an ADI of 0 - 75 mg/kg bw/day) and by the Codex Alimentarius Commission General Standard on Food Additives – Table 3 at the level of Good Manufacturing Practices (ADI "not specified"). Carrageenan is a permitted food additive that is used at a level of 0.03% as a stabilizer in milk or soy-based liquid infant formulas in the USA. In Europe, carrageenan is permitted in follow-on formulas for infants >4 months old, but not permitted in liquid formula for infants <4 months old. Recently the use of carrageenan in infant formula has been considered by Codex Alimentarius (July, 2007). The safety of carrageenan for use in infant formula is based on an extensive database of standard subchronic and chronic feeding studies in adult rodents and primates and rodent reproduction studies. The safety of the infant formula use is confirmed by a 112-day infant baboon formula feeding study using carrageenan at up to a level of 0.15% carrageenan (McGill *et al*, 1977) and post-market surveillance data in human infants (Sherry *et al*, 1993, 1999). An estimate of carrageenan ingestion at the 0.03% use level in formula was made based on a survey of liquid consumption and body weight data for non-breast fed infants 1-6 months of age (Koletzku *et al*, 2000). Exposure to carrageenan in infant formula is estimated at approximately 20 – 34 mg/kg bw/day using these data or below the European ADI of 0-75 mg/kg bw/day and the level found in a post-market surveillance study in human infants (Sherry *et al*, 1993, 1999). It is concluded that carrageenan is safe for use in infant formula based on existing literature, toxicology and exposure data.

492 EXPRESSION OF CU/ZN SUPEROXIDE DISMUTASE AND CATALASE BY ALTERATION OF PPARγ AND NF-κB DNA BINDING ACTIVITIES.

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Structural similarities of lipophilic compounds to ligands for peroxisome proliferators-activated receptor gamma (PPARγ) maybe linked to gene regulation. Expression of antioxidant enzymes, Cu/Zn superoxide dismutase (SOD) and catalase, are linked to PPARγ and NF-κB activation. Hence, we investigated the effects of lipophilic α-tocopherol (α-T) and isomers of conjugated linoleic acid (CLA) on the expression of the antioxidant enzymes and changes in thiobarbituric acid reactive substances (TBARS) in human umbilical vein endothelial cell (HUVEC) culture. α-T and isomers of CLA treatments resulted in increases in both PPARγ (α-T: 1.4-2.2-fold, p<0.01; CLA isomers: 1.4-1.8-fold, p<0.005) and NF-κB p50 (α-T: 1.3-1.5-fold, p<0.005; CLA isomers: 1.3-1.9-fold, p<0.005) DNA binding activities compared to vehicle control. Also, we found increases in mRNA levels (α-T: 6.0-fold of Cu/Zn SOD, p<0.005, 8.0-fold of catalase, p<0.005; CLA isomers: 5.3-12.4-fold of Cu/Zn SOD, p<0.005; 1.7-14.7-fold of catalase, p<0.005) and protein levels (α-T: 2.3-fold of catalase, p<0.005; CLA isomers: 2.5-8.9-fold of Cu/Zn SOD, p<0.05; 1.6-3.2-fold of catalase, p<0.05) at the lower concentrations of α-T and isomers of CLA. Increases in TBARS levels (α-T: 5.3-fold, p<0.005, CLA isomers: 2.0-3.0-fold, p<0.05) were observed at the lower concentrations of α-T and CLA isomers. Both mRNA and protein levels of these two antioxidant enzymes were positively associated with TBARS levels (p<0.05). Although, α-T may play a role not only in preventing against oxidative stress by scavenging free-radicals and superoxide, it may also modulate the expression of the endogenous antioxidant enzymes through PPARγ and NF-κB in HUVEC. Similar mechanisms in gene expression may exist for CLA. Overall, the α-T or CLA-mediated gene expression is either stimulatory or inhibitory, depending on their oxidative status or concentrations.

493 EFFECTS OF GACINIA CAMBOGIA EXTRACT (HYDROXYCITRIC ACID) ON SERUM SEX HORMONES IN OVERWEIGHT SUBJECTS: A DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED TRIAL.

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(-)-Hydroxycitric acid (HCA), an active ingredient extracted from the Garcinia cambogia fruit rind, has been commonly used as a dietary supplement for weight management. Given the controversy over HCA related testicular toxicity in animal studies, we investigated changes in serum sex hormones levels as an extension of our previous double-blind placebo controlled trial in human subjects, in which 44 participants received either G. cambogia extract (1667.3 mg/day; 1000 mg HCA/day) or placebo for 12 weeks. The study was carried out as per the Helsinki Declaration's ethical guidelines and was approved by the Institutional Review Boards of FANCL Corporation, Maebashi Hirosegawa Clinic (Gunma, Japan), and Ono Clinic (Osaka, Japan).Compared to the placebo group, administration of the extract did not significantly alter the serum testosterone, estrone, and estradiol levels. Similarly, hematology, serum triacylglycerol and serum clinical pathology parameters did not reveal any significant adverse effects. The results of this preliminary investigation indicate that ingestion of G. cambogia extract at dose levels commonly recommended for human use does not affect serum sex hormone levels and blood parameters.

494 SAFETY ASSESSMENT OF SDA SOYBEAN OIL.

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Long chain omega-3 polyunsaturated fatty acids (LC-PUFAs) have been clinically shown to reduce the risk of cardiac mortality. Fish oils are a traditional dietary source of LC-PUFAs, namely, EPA (20:5 n-3 eicosapentaenoate) and DHA (22:6 n-3 docosahexaenoate). With biotechnology, it is now possible to produce an omega-3 fatty acid (FA) in vegetable oils that, when consumed, is converted to EPA. Stearidonic acid (SDA) (18:4 octadecatetraenoate) has been produced in soybean oil (20-30% FA) through the introduction of genes for delta 6 and delta 15 desaturases. These enzymes facilitate conversion of ALA (α-linolenate), an endogenous FA in soy oil, to SDA. Humans convert dietary SDA, in part, to EPA. The supplementation of food with fish oil LC-PUFAs is limited by their instability, potential to impart off-flavors and declining worldwide availability of fish. SDA has one less double bond than EPA and SDA oil has flavor and stability characteristics similar to conventional soybean oil. SDA has a history of consumption since it is a natural component (2-5% FA) of fish oils. However, the projected dietary intake of SDA in soybean oil will exceed existing intakes. Therefore, a 28 day toxicity study in rats was undertaken. Rats convert SDA, in part, to EPA, based on both published studies as well as a 6 week diet study with rats fed 5 grams/kg BW SDA soybean oil. In the 28 day toxicity study, rats were gavaged with 0, 0.3, 1.0 or 3.0 mL/kg of 20% SDA soybean oil in a constant total volume of 3 mL/kg/day (the balance adjusted with soybean oil). There were no deaths and no differences between SDA and control treated rats in body weight, food consumption, organ weights, clinical and microscopic pathology findings. The highest dosage tested was considered to be the NOEL, and provides a margin of exposure 25X higher than anticipated human dietary exposure of SDA when added to foods.

495 EFFECTS OF T-2 TOXIN ON HEPATIC DRUG METABOLIZING ENZYMES AND THE BINDING ACTIVITY OF AFLATOXIN B1 TO DNA IN RATS.

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T-2 toxin is one of the trichothecenes produced by Fusarium species, such as F. sporotrichioides. Since it is known to have the highest toxicity among all trichothecenes, we are concerned about the adverse effects on human health through the consumption of food and feeds contaminated with this toxin. T-2 toxin is a potent inhibitor of eukaryotic protein and DNA synthesis and causes breakage of cellular DNA. The toxin is also known to be a compound that induces immuno-toxic

diseases, such as alimentary toxic aleukia of man. Recently multiple-contamination of mycotoxins, such as trichothecenes and aflatoxins, has been detected in cereals and this raises a new problem, namely whether immunotoxic mycotoxin promotes tumor formation induced by aflatoxins. Aflatoxin B1 is a known genotoxic carcinogen produced from the fungus, Aspergillus. Based on the mechanism of cancer induced by aflatoxin B1, drug metabolizing enzymes in liver, such as chytochrome P450 (CYP 3A4), have been shown to catalyse the formation of aflatoxin metabolite, which is able to bind to DNA, inducing mutations. The immuno-suppressive induction of other mycotoxins can accelerate tumor formation.

In this study, we examined the effect of T-2 toxin on hepatic drug metabolizing enzymes with regards to aflatoxin metabolism and the binding activity of aflatoxin to DNA using rats. A single administration of T-2 toxin increased CYP3A4 activity at 24 hr post administration. The binding assay with ^{3}H- aflatoxin B1 with DNA in vitro and in vivo revealed that T-2 treatment increased the DNA binding activity to aflatoxin.

Therefore, we demonstrated that an immune-toxic mycotoxin, such as T-2 toxin, affects tumor formation induced by aflatoxin B1.

496 HEAT STABILITY OF ANTIBIOTICS CHARACTERIZED BY CHANGES IN CHROMATOGRAPHIC PROFILES, ULTRAVIOLET SPECTROMETRY, ANTIMICROBIAL ACTIVITY AND GENOTOXICITY.

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Heat stability of antibiotics was traditionally accessed either by the decreasing antimicrobial activity to susceptible microbes or by the quantitative reduction of drug concentrations before and after heating treatments. The relationships between the structural degradations of antibiotics after heating and their antimicrobial activity have not been well investigated. In addition, whether or not these heated compounds generate altered genotoxicity that could contribute to mutagenicity of bacteria was not clear. Therefore, 17 antibiotics from major antibiotic classes, including tetracyclines, β-lactams, sulfonamides, aminoglycosides and lincosamides were examined for their heat stability by characterization of the changes in the drugs' chromatographic profiles/ultraviolet spectrometry after heating treatment and compared to the corresponding antimicrobial activity in both gram positive and gram negative bacteria. After heating of the antibiotics for 15 min, the reduction of main peak size was as high as 76% (100°C) and 83% (121°C), while up to 6 new peaks were generated in 10 of the 17 tested antibiotics. Higher temperature was associated with more extra peaks formation and wider spectral shifts, contributing to an increased MIC ranging from 2-1024 folds. However, the structural degradation of main peak did not always correlate with or was proportional to the reduced antimicrobial activity, suggesting that degradation products might contain various degrees of antimicrobial activity. Although the mutagenicity of the degraded parent/new products remains in study, the preliminary results suggested that it is possible the degradation products formed by heating process could generate unpredictable toxicities and can not always be assumed safe. The heat stability of drug residues in the milk or meat will also be investigated.

497 EFFECTS OF SYNEPHRINE, BITTER ORANGE, AND CAFFEINE ON PHYSIOLOGICAL AND CARDIOVASCULAR VARIABLES IN FEMALE SPRAGUE-DAWLEY RATS.

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The purpose of this study was to determine acute physiological and cardiovascular effects of synephrine (S, 90% extract), bitter orange (B, 6% extract equivalent to S), and caffeine (C) in rats. Transmitters monitored temperature, motor activity, blood pressure, and electrocardiography for 5 days in young female SD rats that were gavaged once daily with the vehicle (0.25% methyl cellulose). Subsequently, rats received a daily dose of either B or S at 0, 10, or 50 mg/kg body weight with and without C (25 mg/kg) for 4 weeks. Data were collected prior to dosing and at 0.25, 0.5, 1, 2, 4, and 6 hours after dosing. For activity, QA interval and systolic, mean, and pulse blood pressure variables, there were statistically significant C, B, S, CxB, and CxS effects present at 2 to 6 time points after dosing. Similarly, C, B, CxB, and CxS effects were present for diastolic pressure and temperature, and C, B, S, and CxS effects were found for heart rate. In addition, for all physiological variables except heart rate, a significant linear dose trend in B and B+C was present at 2 to 6 time points after dosing. B, S, B+C, and S+C had a greater effect on blood pressure (increase, +) than on temperature (+), activity (+), heart rate (+), and QA interval (decrease, -). At 0.25 hours after dosing, mean systolic pressure values were 136.6, 137.7, 140.3, 137.0, 138.1, and 146.2 mm/Hg for B0, B10, B50, B0+C25, B10+C25, and B50+C25 dose groups, respectively. B+C and S+C had greater effects than B and S alone. B and B+C at 50 mg/kg had greater effects than S and S+C at 50 mg/kg. B, S, B+C, and S+C decreased the QA interval (increased cardiac contractility) but they had no significant effect on body weight. Abnormalities such as hypothermia, ataxia, prolongated QT intervals, and partially inverted T waves were also found. These results suggest that B and S with and without C may have adverse physiological effects.

498 EFFECT OF DEOXYNIVALENOL ON LPS SIGNALING IN MACROPHAGE.

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Trichothecene mycotoxins produced by Fusarium fungi have been detected in grains including wheat and corn, and are one of the contaminants in cereal-based food. Two 8-ketotorichothecenes, deoxynivalenol (DON) and nivalenol (NIV), which are produced by Fusarium graminearum were found at higher levels in cereals harvested in Japan. Recently, it have been reported that Toll-like receptors, which recognize pathogen-associated molecular patterns, play an important role in innate immunity. It is known that DON causes immune dysfunction, therefore we explored the effect of DON and NIV on macrophage activation in response to lipopolysaccharide (LPS), a component of gram-negative bacterial cell wall, which strongly induced productions of inflammatory cytokines through TLR4. LPS signaling via TLR4 is transduced by two pathways: the MyD88-dependent and -independent pathways. In this study, the signaling of MyD88-dependent and -independent pathways were evaluated by NF-κB-dependent reporter activity and INF-β promoter reporter activity, respectively. The mouse macrophage-like cell line RAW264 stably carrying an NF-κB dependent reporter gene or an INF-β promoter reporter gene was stimulated with LPS in the presence of DON or NIV. DON and NIV inhibited both of the reporter activities in a concentration-dependent manner and stronger inhibitory effect on the INF-β promoter reporter activity was observed with DON. This result suggests that DON selectively inhibits LPS-induced MyD88-independent signaling. We are now investigating the effect of DON on LPS-induced inducible nitric oxide synthase expression, which is regulated by IFN-β promoter in macrophage.

499 APPLICATION OF A TOXICOLOGICAL SURROGATE EVALUATION FRAMEWORK: ALKYL AMMONIO ACETATES AS A CASE STUDY.

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The evaluation of potential human health risk from exposure to chemicals relies surprisingly often on data developed for structurally-related chemicals that are predicted to have similar toxicological properties. This approach is used frequently in the context of hazard screening for new chemical development and is embodied in the use of read-across approaches within the U.S. EPA High Production Volume Chemical Challenge program and in many other risk and toxicity applications. Despite the long history of reliance on this analogue approach, very little formal guidance is available for identifying or validating the selection of toxicological surrogates or for defining and documenting the adequacy of a proposed chemical analogue. In response to this shortcoming we have developed a structured data evaluation framework that provides an approach for weighing alternative lines of evidence pertinent to assessing toxicological analogy. Lines of evidence that are formally addressed in the framework are both qualitative and quantitative in nature. Decision criteria were developed for the following: consideration of visual inspection, assessing the presence of potentially reactive groups, assessing toxicokinetic considerations, using data clustering and regression approaches, and the incorporation of output from multiple published SAR models. Strategies for assessing similarities in hazard categories as well as techniques for estimating toxic potency are addressed in the framework. We demonstrate the application of each of the framework elements for assessing the toxicity of a series of alkyl ammonio acetates as a case study.

500 EFFECTS OF OCCUPATIONAL EXPOSURE TO CHEMICALS ON HEARING - A WEIGHT OF EVIDENCE APPROACH.

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There is accumulating epidemiological evidence that exposure to some solvents, metals, asphyxiants and other substances in humans is associated with an increased risk of acquiring hearing loss. There are no regulations that require hearing monitoring of workers who are employed at locations in which occupational exposure to potentially ototoxic chemicals occurs in the absence of noise exposure. This project was undertaken to develop a toxicological database allowing the identification of

possible ototoxic substances present in the work environment. Critical toxicological data were compiled for chemical substances included in the Quebec Occupational Health Regulation. The data were evaluated only for realistic exposure concentrations up to the short-term exposure limit or ceiling value or five times the 8-h time weighted average occupational exposure limit for human data and up to 100 times the 8-h TWA OEL or ceiling value for animal studies. In total, 180 studies covering 29 substances were evaluated using a weight of evidence approach. For the majority of cases where potential ototoxicity was previously proposed, there is a paucity of toxicological data in the primary literature. Human and animal studies indicate that ethyl benzene, lead, styrene, toluene and trichloroethylene are ototoxic and carbon disulfide, n-hexane and xylene are possibly ototoxic at concentrations that are relevant to the occupational setting.

501 SAFE DRINKING WATER FROM LONG-LIFE, POINT-OF-USE HOUSEHOLD TREATMENT BASED ON BROMINE CHARGING OF POLYSTYRENEHYDANTOIN BEADS (HALOPURE).

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Problem: Point-of-use (POU) drinking water solutions are not widely accepted in resource-poor countries due to compromise on one or more features: good taste; ease of use and maintenance; low cost; microbiological effectiveness; residual disinfectant to prevent recontamination in storage; absence of offensive odors or harmful contaminants. Hypothesis: A POU system using HaloPure® can effectively reduce microbiological contaminants to create acceptable drinking water. Methods: A POU household drinking water system was developed using HaloPure®, an N-halamine based, derivatized polystyrene disinfection medium, which can be brominated or chlorinated for contact biocidal activity in a single pass flow of water. Biocidal effectiveness is maintained by a replaceable activator tablet, a slow-eluting bromine source to repopulate binding sites on the HaloPure® beads, extending routine use for years. When used in conjunction with a typical household gravity-feed ceramic candle water filter canister, the POU device adds disinfecting function to basic particle filtration. Microbiological challenges were performed and disinfection byproducts measured. Results: The device achieves 6 log reduction of bacterial (*Klebsiella* terrigena) and 4 log reduction of viral (polio and rotavirus) contaminants, as well as 4 log reduction of protozoan cyst contaminants when using cyst-reducing ceramic candles. Associated disinfection byproducts are below USEPA MCLs: TTHM were 0.072 mg/L and HAA5 were 0.010 mg/L in the first 400 L of treated water, with lower levels subsequently. Mean residual bromine was 0.50 mg/L over the course of more than 3400 L treated water, achieving storage protection, but low enough to avoid offensive odor or taste. For the drinking water needs for a family of five, the total maintenance cost of inexpensive ceramic prefilters and activator tablets is estimated at $0.019 USD per day. Conclusion: This POU device has the potential to achieve widespread use with a significant public health impact in resource-poor settings.

502 PROPOSAL FOR SAFETY EXPOSURE LEVEL OF NITROBENZENE THROUGH FOODS AND DRINKING WATER.

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Nitrobenzene is produced in high volume around the world, and the major use is an intermediate in the synthesis of a variety of nitroaromatic compounds, primarily aniline. Because of concern that humans have been exposed to this chemical through foods and drinking waters, the toxicity assessment should be conducted to derive the safety level such as tolerable daily intake (TDI). In the repeated-dose toxicity studies, nitrobenzene principally caused methemoglobinemia and histopathological changes in the liver and kidney. Since no chronic oral toxicity study has been reported, the TDI was established based on the results of chronic inhalation toxicity study after the route-to-route conversion. The common toxicity observed in short-term oral and inhalation studies support this dosimetric adjustment. By applying an uncertainty factor of 100 for inter- and intra-species variations to the lowest $BMDL_{10}$ (1.05 mg/kg bw/day) derived from 2-year inhalation study using rats, the TDI of 10.5 μg/kg bw/day was estimated for repeated-dose toxicity of nitrobenzene. For the reproductive/developmental toxicity, only slight decrease in pup weight was observed at the lowest dose of 20 mg/kg bw/day, which induced overt maternal toxicity, in an oral combined repeat dose and reproductive/developmental toxicity study using rats. Nitrobenzene showed negative results in classical mutagenic tests. However, the genotoxic potential could not be ruled out because positive results were given in some recent studies. The potential carcinogenicity of inhaled nitrobenzene was evidenced by 2-year mice and rat studies, demonstrating a spectrum of benign and malignant neoplasias. Based on the combined incidence of liver, thyroid, and kidney tumors in rats, a dose of 0.27 μg/kg bw/day at the risk of 10^{-5} was estimated by dividing the $BMDL_{10}$ (2.7 mg/kg bw/day) with 10,000. In conclusion, oral safety levels of nitrobenzene for human life-time were estimated as 10.5 and 0.27 μg/kg bw/day for non-cancer and cancer endpoints, respectively.

503 EVALUATION OF CLIMBAZOLE GENOTOXICITY IN A BATTERY OF *IN VITRO* AND *IN VIVO* MUTAGENICITY TESTS.

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Climbazole is an imidazole-type antifungal agent that can provide anti-dandruff benefits when incorporated into a shampoo matrix. A series of genotoxicity studies were performed with climbazole to support the human safety of this compound. Three in vitro studies were conducted to determine gene mutations in bacterial (Ames) and mammalian cell systems (Mouse Lymphoma Assay) and clastogenicity (Micronucleus) in human peripheral blood lymphocytes. Climbazole was not mutagenic to S. typhimurium or E.coli strains and did not induce micronuclei in human lymphocytes. In the Mouse Lymphoma Assay, climbazole was negative (non-mutagenic) with and without metabolic (S-9) activation with a 4-hour exposure and positive without activation with a 24-hour exposure at concentrations of 15 and 17.5 μg/mL. There was an increase in small colony mutants consistent with chromosome damage. To determine the biological relevance of this in vitro response, in vivo studies (Mouse Micronucleus and Rat Unscheduled DNA Synthesis (UDS)) were conducted. No evidence of chromosomal alterations was seen in the Micronucleus test after single oral administration of up to a maximum tolerated dose (MTD) of 150 mg/kg climbazole. Evaluation of the genotoxicity profile of climbazole in the UDS assay showed no evidence of DNA damage up to the MTD of 200 mg/kg. To demonstrate that upon oral administration, climbazole was bioavailable; a toxicokinetic study was performed with [14C]-climbazole. [14C]-climbazole was detected in plasma as early as 15 minutes after oral administration (20.42 μg-equiv/g plasma) and a peak concentration was observed at 8 hours (62.96 μg-equiv/g plasma). These blood levels that exceed the concentrations that were positive in vitro (15 and 17.5 ug/ml) support the conclusion that climbazole does not present a genotoxic risk in vivo. Further, these data are consistent with genotoxic evaluations for other imidazole antifungals that as a class are largely non-genotoxic. Collectively, these data indicate that climbazole does not present a carcinogenic risk to humans.

504 21-DAY DERMAL TOXICITY STUDY OF EUCALYPTUS ESSENTIAL OIL IN FEMALE RATS.

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Eucalyptus oil has been widely used in treatment of bacteria infection or prevention. Some studies reported that Eucalyptus essential oil inhibit the growth of s. typhosa, p. morgani, b. brevis and m. citreus. For aromatherapy, Eucalyptus oil is also applied to human body either by inhalation or massage. This study is to evaluate the potential adverse effects of Eucalyptus oil by dermal exposure for consecutive 21 days.

Four groups of female Sprague-Dawley rats were dosed of pure Eucalyptus essential oil by dermal administration at the dose level of 0.5, 1.0, 1.5 mL/kg/day. Rats were dosed 6 hours a day with an area of 5x5 cm2. The assessment of toxicity was based on mortality, clinical observations, body weight, food consumption, organ weight, clinical pathology and gross evaluation.

Animals were all survived to the end of the study. No abnormal clinical observations were found during the in-life period. Body weight and food consumption were not significantly different from control values. Parameters of clinical pathology, including urine analysis, hematology and serum chemistry, were not altered by Eucalyptus essential oil treatment. For gross observation, no exposure-related gross lesions were noted at the exposure sites (skin) at necropsy. However, 1/5, 2/5, and 3/5 of low, middle, and high dose group animals were found pale and protruded area with the size of 0.6-0.7 cm in liver, respectively.

The common daily human use of Eucalyptus oil is about 0.3 mL for an adult (60 kg subject). Based on our results, rats received dose of 0.5 mL/kg/day of Eucalyptus essential oil for 21 days via dermal exposure indicated some liver gross lesions. The dose is about 100 folds over the recommended human dose basing on a conversion of body weight. Further histopathological evaluation will be conducted to reveal if there is any the microscopic lesions of these tissues.

505 CUTANEOUS LOCAL TOLERANCE OF DIPHOTéRINE® IN HUMAN VOLUNTEERS AFTER A UNIQUE OCCLUSIVE APPLICATION DURING 48 HOURS.

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Diphotérine® is an amphoteric, water-soluble, polyvalent, slightly hyptertonic, non-toxic, non-sensitizing, non-mutagenic, and non-irritating compound for active eye/skin decontamination. Its chemical and physical properties allow a quasi polyvalent rinsing of chemical splashes with a rapid return to a physiological state. It may be used by both dermal and ocular application.

The local tolerance caused by Diphotérine® has already been evaluated on rabbits following a single, semi-occluded or non occluded application to scarified and non-scarified skin on rabbits. A semi-occluded application was used to mimic the contact of wet clothes soaked with Diphotérine® and a non-occluded application to imitate a normal application of Diphotérine® on skin. No local cutaneous reaction or toxic effects were observed.
Reported here is the study of acute skin tolerance after a single occlusive patch test during 48h. The conditions of the test were extreme compared to normal Diphotérine® use which is external washing lasting only a few minutes.
The study was performed on 55 adult following Ethical Committee approval and patient consent. The ages ranged from 18 to 70 years old. 0.02 ml of pure Diphotérine® was applied on the arm and maintained during 48 hours with an occlusive bandage. The clinical evaluation was made half an hour after bandage removal and is based on the intensity of the appearance of erythema, edema, papule, vesicles and bulla, graduated from 1 to 4. The total sum of the scores, divided by the number of volunteers defines the average irritation index which allows classifying of the product. The average irritation index for Diphotérine® is: 0.00.
Diphotérine®, applied in its pure form, can be considered as non-irritating after an application during 48 hours on 55 volunteers.

506 SUBCHRONIC TOXICITY OF DIMYRCETOL IN RATS.

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The subchronic toxicity of dimyrcetol (CAS Nos. 18479-58-8; 25279-09-8), a widely used fragrance ingredient, was evaluated in a 13-week oral toxicity study in rats. Male and female rats (10/sex/dose) were gavaged once daily with dimyrcetol in corn oil at dose levels of 0, 10, 50, 500 or 1000 mg/kg/day. Control animals received the vehicle alone. Observations included morbidity and mortality assessments, body weights, food consumption, estrous cycle and ophthalmology. Hematology, blood chemistry and urinalysis were evaluated at weeks 7 and 13. A complete necropsy was conducted on all animals. There were no unscheduled deaths and there were no clinically observable signs of toxicity. Statistically significant reductions in body weight gain were observed in both sexes at 1000 mg/kg/day. A statistically significant increase in absolute and relative liver and kidney weights was noted in both sexes in the 2 highest dose groups. Elevated liver weights were also noted in males in the 50 mg/kg/day dose group. Significant increases in plasma urea and creatine were noted in males treated with 50, 500 or 1000 mg/kg/day. There were no treatment-related effects on female estrous cycles. No macroscopic abnormalities were detected at necropsy. In the 2 highest dose groups, statistically significant reductions in platelet count, activated partial thrombastin time and albumin/globulin ratios and statistically significant increases in cholesterol, aspartate aminotransferase and alanine aminotransferease were noted. A statistically significant greater incidence and/or severity of groups of basophilic tubules and/or globular accumulations of eosinophilic material were observed in males in all dose groups. This finding is consistent with the presence of hydrocarbon nephropathy resulting from excessive accumulation of alpha-2-microglobuin and was not considered relevant. Statistically significant tubular necrosis was also observed in males in the 2 highest dose groups. Based on these findings, it can be concluded that the no-observable-effect-level (NOEL) for females is 50 mg/kg/day and that the no-observable-adverse-effect-level (NOAEL) for males is 10 mg/kg/day.

507 NOVASIL CLAY DOES NOT AFFECT BIOAVAILABILITY AND UTILIZATION OF VITAMINS A AND E AND NUTRIENT MINERALS IN GHANAIANS AT HIGH RISK FOR AFLATOXICOSIS.

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To assess the potential interference of NovaSil (NS) clay with micronutrients in humans, vitamins (Vits) A and E and minerals (15 nutrient and 15 non-nutrient minerals) were measured in serum samples from a 3-month intervention trial with NS. Participants (177) were randomly divided into three groups that received 3.0g NS/day (high dose, HD), 1.5g NS/day (low dose, LD), or placebo (PL). Levels of Vits A and E in serum were comparable among the three study groups at baseline, 1-month and 3-months of NS intervention. A gender-stratified non-parametric mixed-effect model analysis showed no significant effects of dose and dose-time interaction for levels of Vits A and E; however, a significant increase in the levels of Vit E was detected in the male participants over the period of the intervention. No significant differences were found in levels of the nutrient and non-nutrient minerals between the HD and PL groups at baseline and 3-months of NS intervention, except for strontium (Sr) levels. Strontium was significantly increased ($p < 0.001$) in the HD group (male: 113.65 ± 28.00 μg/L; female: 116.40 ± 24.26 μg/L) compared to the PL group (male: 83.55 ± 39.90 μg/L; female: 90.47 ± 25.68 μg/L) following the 3-month intervention with NS. These results, combined with safety and efficacy data, suggest that NS clay is highly effective in reducing aflatoxin exposure and does not affect the bioavailability and utilization of important vitamins and nutrient minerals in humans.

508 METHYL TERTIARY BUTYL ETHER (MTBE) 13-WEEK DRINKING WATER STUDY IN WISTAR RATS.

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MTBE has been found to be present in a variety of environmental media including groundwater and thus presents the need for information on the toxicology of this compound when administered in drinking water. Male and female Wistar rats were administered MTBE via drinking water at concentrations of 0, 0.5, 3, 7.5, and 15 mg/ml for 13 wks. Body weights, clinical signs, and food and water consumption were monitored weekly. Urine was collected from males and analyzed at 4 and 21 d. Cell replication in the kidney was assessed at 1, 4, and 13 wks. Complete tissue histopathology was performed on animals exposed for 13 wks. Serum hormone levels were assayed after 28 d exposure. Body weights in males of the 7.5 and 15 mg/ml groups were less than control at 13 wks exposure and were unaffected in females. MTBE exposure resulted in decreased water consumption in all dose groups and unchanged food consumption, in both sexes. Males had elevated urine specific gravity and osmolality. Serum hormone levels were unchanged by treatment in either sex. Kidney wet weights were elevated in males and females exposed to 7.5 and 15 mg/ml. Cell replication of kidney cortical epithelial cells was unchanged in females but was elevated in males of the 15mg/ml group at 4 wks. Renal tubular cell regeneration was noted in males exposed to 15 mg/ml, at 13 wks of exposure. Quantitative alpha-2u globulin (α2u) levels in kidney were elevated in males of the high dose group at 1 and 4 wks of exposure. The average (± sd) daily MTBE dose for males was 37 ± 10, 209 ± 61, 514 ± 142, and 972 ± 288 mg/kg/day and for females 50 ± 12, 272 ± 51, 650 ± 142, and 1153 ± 191 mg/kg/day, for 0.5, 3, 7.5, and 15 mg/ml doses, respectively. The results of the present study demonstrate depressed water consumption in male and female rats of all MTBE dose groups and toxicity in males consisting of depressed body weights and effects in the kidney including incipient α2u nephropathy in males exposed to 7.5 and 15 mg/ml. [This study was sponsored by Shell Oil Company, Chevron USA, Inc., and ExxonMobil Corporation].

509 SCREENING LEVEL RISK EVALUATIONS FOR OCCUPATIONAL EXPOSURES TO CHLOROPRENE DIMERS.

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Chloroprene monomer (CD, 2-chloro-1,3-butadiene) is thermally unstable and can spontaneously dimerize. Low levels of CD dimers may occur in finished polychloroprene rubber articles. The potential risks from inhalation of CD dimers during manufacture of polychloroprene and from use of polychloroprene surgical and exam gloves were assessed in this study. CD dimer exposure was evaluated by extraction studies on several commercially-available gloves using a large excess of synthetic sweat. Individual dimers were identified using a GC/MS analytical method (LOQ of ~10 ppb). Sweat extractable CD dimer content was 0.011 μg/g for surgical gloves (n=1), and 0.30–0.57 for exam gloves after 1 hr (n=2). Although there are no known health-based exposure limits for CD dimers, a 2 ppm exposure limit for CD was used as the occupational health benchmark based on analogy to CD and available toxicity data on CD dimers. Measured workplace air concentrations of CD dimers were approximately 100-fold below this 2 ppm limit. To estimate allowable dermal exposure, a systemic dose equivalent to inhaling 2 ppm CD dimers over the workshift was calculated (140 mg/day). Based on the amount of CD dimers extractable in sweat from gloves worn for 6 hr, the estimated daily dose of CD dimers (assuming 100% absorption) would be 0.00010 mg/d from surgical gloves and 0.0038 mg/d from exam gloves. These estimated doses are about 1,400,000- and 38,000-fold, respectively, less than the dermal-equivalent occupational limit for CD dimers. Time course studies showed essentially all sweat-extractable CD dimer was extracted in the first minute, so an assessment was performed to account for multiple exam glove changes. In this scenario, the estimated CD dimer dose was 0.14 mg/d, a value 1000-fold less than the projected allowable dermal-equivalent exposure limit. The exposure estimates used in these studies (based on a limited number of samples and numerous conservative assumptions of potential exposure) indicate that risk from exposure to CD dimers in these occupational scenarios is minimal.

510 NASAL EPITHELIAL LESIONS IN RATS FOLLOWING AN ACUTE INHALATION EXPOSURE TO NAPHTHALENE VAPOR AT LOW CONCENTRATIONS.

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Inhalation exposures of F344 and SD rats to naphthalene (NP) vapor have resulted in nasal lesions. In order to examine the differences in NP-induced effects in the nasal cavity between F344 and SD strains, and to study the exposure-response relationships for nasal epithelial effects at low NP concentrations, a system to generate and characterize NP vapor at 30, 10, 1, 0.3, and 0.1 ppm was developed and tested. NP vapor was generated by diluting sublimed solid NP into the exposure chamber air supply. NP concentration was measured by gas chromatography. Aerosol testing was performed to verify that solid naphthalene particles (potentially formed by the condensation of naphthalene) were not present. The next day after a single 6-hour whole body exposure, groups of male and female F344 and SD rats were killed and nasal tissues were taken for histopathological analysis. Respiratory and olfactory necrosis was observed at the 10 and 30 ppm exposure concentrations in male and female, F344 and SD rats. Olfactory epithelium necrosis was observed in SD and F344 rats at 1 ppm, whereas the SD rats appeared to be more sensitive, with minimal olfactory epithelium damage in some animals as low as 0.1 to 0.3 ppm.

511 IMPAIRED GLUCOSE TOLERANCE IN RODENTS ADMINISTERED HIGH DOSES OF GLUCOSAMINE ARE NOT RELEVANT TO HUMANS.

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Glucosamine (Glcn) is commonly used in the management of osteoarthritis. Despite overwhelming evidence that its use is safe, concerns have been raised that long-term use may induce or worsen diabetes, a contention based entirely on rodent and in vitro studies. It is believed that Glcn may impair glucose (Glc) tolerance by interfering with the hexosamine biosynthesis pathway. In theory, exogenous Glcn could alter Glc metabolism by entering the pathway where endogenous Glcn is produced, impairing the proposed function of the pathway as a nutrient sensor. An unappreciated mechanism by which Glcn could induce Glc intolerance is through the inhibition of glucokinase. Upon entry into the cell, Glc is phosphorylated by glucokinase ensuring that a constant gradient is maintained for Glc transport. Glcn, via competition for glucokinase, could impair Glc uptake, by reducing Glc phosphorylation. However, Glc intolerance and insulin insensitivity have not been convincingly demonstrated in humans, and the mechanism for the species differences is unknown. The results of our investigation indicate that differences in the affinity of glucokinase for Glcn and Glc is a probable explanation for the species specific effects. In the rat, the affinity of glucokinase for Glc and Glcn is 8 and 5 mM respectively, a differential that would slightly favor Glcn binding. The preference of rat glucokinase for Glcn is supported by in vitro studies, where the phosphorylation of Glcn by glucokinase is unaffected by physiological concentrations of Glc (5mM). In contrast, in humans the affinity of glucokinase for Glc is 10-fold higher than that of Glcn. This observation suggests that the effect of Glcn on glucose metabolism in animals is not relevant to humans. Given the limited oral bioavailability of Glcn, and the 10-fold higher affinity of glucokinase for Glc over Glcn, it would be virtually impossible to achieve plasma levels of Glcn that would alter Glc metabolism in humans. Thus, further studies investigating the ability of Glcn to cause or worsen diabetes in humans are clearly not warranted.

512 PRELIMINARY PALATABILITY TESTING OF IONIC LIQUIDS IN WISTAR HAN RATS AND B6C3F1 MICE.

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Ionic liquids (ILs) have emerged as potential green alternatives to traditional organic solvents. Their structural flexibility and a varied array of cations and anions make it possible to design ILs for specific applications. Ionic liquids are highly soluble in water which could increase exposure to the environment. However, literature describing the toxicity of ionic liquids is limited. For these reasons several ionic liquids have been selected for toxicological evaluation by the National Toxicology Program (NTP). This work describes 14-day palatability studies in rats and mice given drinking water containing one of four ionic liquids; 1-butyl-1-methyl-pyrrolidinium chloride ([bmpy]Cl), 1-butyl-3-methyl-imidazolium chloride ([bmim]Cl), N-butyl-pyridinium chloride ([NBuPy]Cl) and 1-ethyl-3-methyl-imidazolium chloride ([emim]Cl). The drinking water dose levels for [bmpy]Cl, [bmim]Cl and [NBuPy]Cl were 3, 10 and 30 mg/ml and for [emim]Cl were 3, 5, and 8 mg/ml. Both rats and mice given the 10 or 30 mg/ml dose of [bmpy]Cl, [bmim]Cl or [NBuPy]Cl, and rats given the 3 mg/ml dose of [bmim]Cl, demonstrated clinical signs, decreased food consumption and decreased body weight within the first three days. These groups were returned to untreated drinking water on days 4 and 7, respectively, due to body weight loss greater than 10 percent of their original weight for 3-5 consecutive days. The body weights of these animals recovered once returned to untreated drinking water. No adverse effects were observed in rats and mice in the 3 mg/ml group for [bmpy]Cl and [NBuPy]Cl. No effects were observed in either rats or mice on any parameters with any drinking water concentration of [emim]Cl. The results from these limited studies suggest that the palatability of ionic liquids varies with chemical structure and that future toxicology studies using drinking water exposure should utilize concentrations less than 10 mg/ml.

513 QUARTZ-CONTAINING CERAMIC DUSTS: *IN VITRO* SCREENING OF THE CYTOTOXIC AND GENOTOXIC POTENTIAL OF 5 FACTORY SAMPLES.

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Inhalation of respirable crystalline silica (RCS) may lead to chronic inflammation, silicosis and cancer. Considering ubiquitous occurrence, diverse use and complex characteristics of quartz toxicity, compre¬hensive physicochemical and biological data sets are strongly needed to allow differentiation of various quartz varieties. In the European Collective Research Project SILICERAM an in vitro screening battery was established and used to evaluate the cytotoxic (LDH-release, MTT-assay), genotoxic (Comet-assay) and inflammatory (PGE2-liberation, TNF-alpha mRNA expression) potential of RCS-containing dusts in primary rat alveolar macrophages and NR8383 cells. To judge quartz-specificity, aluminium lactate (AL), a well-known quencher of quartz-specific biological effects, was used. Within the project, 5 ceramic dust samples, collected at various industrial sectors/manufacturing steps, i.e. brick (B; quartz content: 7.8%), tableware granulate (TG; 5.8%), tiles (TI; 8.1%), tableware cast (TC; 3.1%) and refractory ware (RW; 3.7%) were screened. Except TC, all dusts mediated only moderate, mostly not quartz-specific, increase in LDH-levels, compared to Al2O3 as particulate negative control. The MTT-assay demonstrated the mainly quartz-independent rank order: RW>TG>TI>B>TC. In the Comet-assay B and TI exhibited the highest DNA-damaging potential, followed by TG, TC and RW. About 50% of the TG, B, and TI effects were quartz-related, whereas TC and RW showed values of 20 and 40%. All factory dusts induced higher PGE2-liberation than Al2O3 (B>TC>TG>TI>RW). TNF-alpha mRNA expression was only significantly altered by TG, DQ12 (quartz-related) and TC (no AL effect). In conclusion, the in vitro battery has been found to be an adequate approach to screen and differentiate the multifactorial toxic potential of quartz-containing ceramic dusts.

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514 QUARTZ-CONTAINING CERAMIC DUSTS: *IN VIVO* STUDY ON INFLAMMATORY EFFECTS OF TWO FACTORY SAMPLES IN LUNGS AFTER INTRATRACHEAL INSTILLATION IN A 28-DAY STUDY WITH RATS.

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Objective: This 28-day study, part of the European Collective Research Project SILICERAM, aimed at differentiating the biological activity of four quartz species, i.) two quartz-containing materials collected at different ceramic manufacturing sectors (Tableware Granulate, TG; Tableware Cast, TC) versus ii.) a Contrived Sample (CS; 30% quartz, 50% china clay and 20% feldspar) and iii.) ground standard quartz DQ12 (Positive Control; PC). Methods: Total doses of 5 mg per rat of the TG and TC, 1.1 mg of the CS and 0.33 mg of the PC corresponding to 0.29, 0.16, 0.29 and 0.29 mg quartz per rat, respectively, were administered to rats by instillation. Results: Bronchoalveolar lavagate (BAL) analysis showed that the polymorphonuclear neutrophil (PMNs) levels after 3 days were statistically significantly elevated in the TG and PC groups, i.e. 15% and 25% vs. 0.4% in the Vehicle Control (VC). Interestingly, the CS showed 0.6% PMNs only indicating a strong quartz-covering effect by clay and feldspar. At 28 days, the values were 29%, 20%, 7% and 45% for the TG, the TC, the CS and the PC group, respectively. Thus, for the TG and TC groups a moderate response was observed, statistically significantly lower

than the severe inflammation induced by PC. Other BAL endpoints (lactic dehydrogenase, beta-glucuronidase, total protein) showed partial recovery for TG and TC from day 3 to day 28, whereas the PC conclusively showed a progression of effects. Histopathologically, for TG and TC very slight/slight effects after 28 days were detected. In contrast, the PC group exhibited stronger effects. Conclusion: The findings observed resulted in a clear ranking: PC > TG > TC > CS > VC. From a regulatory point of view, these substance-specific toxicological effects of TG and TC may need to be considered when devising occupational exposure limits.
The work was supported by the EC: COLL-CT-2003-500 896.

515 CARCINOGENESIS STUDIES OF CRESOLS IN RATS AND MICE.

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Cresols occur in some plant oils, are combustion or distillation products of wood, coal, and petroleum, and are high production chemicals with potential for occupational and consumer exposure. The three isomers of cresol are used individually or in mixtures as disinfectants, preservatives, solvents, and as chemical intermediates. Carcinogenesis studies were conducted in groups of 50 male F344/N rats and 50 female B6C3F1 mice exposed to a 60:40 mixture of *m* and *p* cresols (*m*-/*p*-cresol) in feed. Rats and mice were fed diets containing 0, 1500, 5000, or 15,000 ppm and 0, 1000, 3000, or 10,000 ppm, respectively. Survival of the exposed groups was similar to that of controls. However, mean body weights were generally lower than controls in the high dose groups, more so in mice than in rats. The decreases appeared to be associated with lack of palatability of the feed. A dose-related increase in the incidence of hyperplasia of the respiratory epithelium of the nasal passages was observed in both rats and mice. The kidney of rats and the forestomach of mice were target organs for carcinogenicity related to cresol exposure. There was equivocal evidence of carcinogenic activity of *m*-/*p*-cresol in male F344/N rats based on a marginally increased incidence of renal tubule adenoma in the group exposed to 15,000 ppm. The increased incidence was not statistically significant, but did exceed the range of historical controls. No increased incidence of hyperplasia of the renal tubules was observed. A significantly increased incidence of hyperplasia of the transitional epithelium of the kidney in these rats was associated with nephropathy. There was some evidence of carcinogenic activity of *m*-/*p*-cresol in female B6C3F1 mice based on an increased incidence of squamous cell papilloma of the forestomach in the group exposed to 10,000 ppm. Although cresols are known contact irritants, there was little evidence of injury to the forestomach of these mice.

516 TOXICITY AND CARCINOGENICITY OF CHROMIUM FOLLOWING CHRONIC ORAL EXPOSURE: DEPENDENCE ON VALENCE STATE AND TISSUE UPTAKE.

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Humans are exposed to hexavalent chromium (Cr VI) by inhalation, and Cr VI is recognized as a human carcinogen by this route. Humans are also exposed to Cr VI in contaminated drinking water. Trivalent chromium (Cr III) is an essential element involved in carbohydrate and fat metabolism, and is commonly found in weight loss supplements as chromium picolinate. Both intra- and extracellular reduction of Cr VI to Cr III can occur. Extracellular reduction is thought be a protective mechanism, while intracellular reduction is thought to be a mechanism of toxicity. The NTP conducted 2-year drinking water studies of Cr VI, as sodium dichromate dihydrate (SDD) and feed studies of Cr III, as chromium picolinate monohydrate (CPM), in male and female F344N rats and B6C3F1 mice. Total chromium was measured in tissues and excreta of male rats and female mice. Following exposure to SDD, there was clear evidence of carcinogenicity in male and female rats and mice, based on increases in squamous cell neoplasms of the oral cavity in rats and in epithelial neoplasms of the small intestine in mice. Reduced body weight gains, decreased water consumption due to poor palatability, a microcytic hypochromic anemia, and histiocytic cell infiltration in the liver, duodenum, and pancreatic and mesenteric lymph nodes were observed in rats and mice; diffuse epithelial hyperplasia of the small intestine was observed in mice. In contrast, there was little to no evidence of adverse effects resulting from CPM exposure. In rats and mice, similar exposures of SDD and CPM resulted in much higher tissue Cr concentrations following treatment with SDD. Collectively, these results demonstrate that Cr VI escapes reduction to Cr III in the stomach, enters cells more readily than Cr III and is significantly more toxic and carcinogenic than Cr III, following oral exposure.

517 TISSUE DISTRIBUTION OF CHROMIUM IN MALE FISCHER 344 RATS AND FEMALE B6C3F1 MICE EXPOSED TO SODIUM DICHROMATE DIHYDRATE IN DRINKING WATER FOR 2 YEARS.

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Hexavalent Chromium (Cr VI) is recognized as a human carcinogen when exposure occurs via inhalation. Recently, a 2-year NTP bioassay of sodium dichromate dihydrate (SDD) in drinking water demonstrated clear evidence of carcinogenicity in rats and mice. As part of the 2-year study, chromium (Cr) levels were measured in excreta and tissues from female mice and male rats. SDD was administered in drinking water at concentrations of 0, 14.3, 57.3, 172, and 516 ppm. On days 4, 11, 180, and 369, up to 10 animals per exposure group were removed from treatment and placed in individual metabolism cages for collection of urine and feces (from 0-24 and 24-48 hours); undosed water and feed were provided *ad libitum*. Liver, kidney, forestomach, glandular stomach, blood and plasma were collected on days 6, 13, 182, and 371. Total Cr in each sample was quantitated by inductively coupled plasma-mass spectrometry. Most of the Cr eliminated was in feces in rats and mice, suggesting that absorption was low. The highest Cr concentrations were found in the kidney, liver and glandular stomach of rats and mice. The concentrations in mouse tissues were generally higher than rat tissues, with liver, forestomach and glandular stomach showing the greatest difference. In both rats and mice, Cr concentrations in erythrocytes and plasma reflected patterns observed in the tissues, but at much lower concentrations. The data from this study will aid in the interpretation of the bioassay results.

518 TISSUE DISTRIBUTION OF CHROMIUM IN MALE FISCHER 344 RATS AND FEMALE B6C3F1 MICE EXPOSED TO CHROMIUM PICOLINATE MONOHYDRATE IN THE DIET FOR 2 YEARS.

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Chromium picolinate monohydrate (CPM), which contains chromium in the trivalent state (Cr III), is a popular dietary supplement, taken primarily for weight loss. As part of a 2-year NTP bioassay to assess the toxicity and carcinogenicity of CPM, total Cr was measured in tissues and excreta of male rats and female mice. CPM was administered in feed at concentrations of 0, 2000, 10000, and 50000 ppm. On days 4, 11, and 180, up to 10 animals per exposure group were removed from treatment and placed in individual metabolism cages for collection of urine and feces (from 0-24 and 24-48 hours); undosed feed and water were provided *ad libitum*. Liver, kidney, forestomach, glandular stomach, blood and plasma were collected on days 6, 13, and 182. Total Cr in each sample was quantitated by inductively coupled plasma-mass spectrometry. Most of the Cr eliminated was in the feces in both rats and mice, suggesting that absorption of Cr III was very low., The highest Cr concentrations were found in the kidneys of rats and livers of mice; kidney Cr concentrations in rats were up to 6-fold higher than in mice. The concentrations in glandular stomach and forestomach did not demonstrate a clear pattern across exposure concentrations or duration. Tissue Cr concentrations generally reached a plateau by 10000 ppm, indicating saturation of absorption or uptake. In general, Cr concentrations in erythrocytes and plasma reflected patterns seen in the tissues, but at much lower concentrations. The data from this study will aid in the interpretation of the bioassay results.

519 MODIFYING EFFECTS OF TROGLITAZONE ON THE VASCULAR TUMORIGENESIS INITIATED WITH URETHANE IN RASH2 MICE.

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The rasH2 mice are hemizygous transgenic mice carrying the human prototype c-Ha-ras gene with its own promoter, and it is well recognized that they are not only susceptible to genotoxic carcinogens but also to some non-genotoxic carcinogens such as peroxisome proliferator-activated receptor (PPAR) α agonists. In our previous study, to evaluate the carcinogenicity of a PPAR γ agonist, troglitazone (TRG), 7-week-old male and female rasH2 mice were fed diet containing 0, 3000 or 6000

ppm TRG for 26 weeks. As a result, an increased incidence of vascular tumors was observed in females of the 6000 ppm group. In order to investigate the mechanism of tumor promoting effects of vascular tumors induced by TRG, female rasH2 mice were fed diet containing 6000 ppm of TRG for 16 weeks after initiation treatment of urethane (UR), histopathological and molecular biological analyses were performed on the vascular tumors of the spleen induced. Vascular tumors were induced in the TRG treated groups, but no significant difference in the incidence was observed between the UR alone group and UR + TRG groups. There was an increasing tendency in the number of positive cells for anti-PCNA antibody in the UR + TRG group. In gene expression analysis, increasing tendencies of VEGF, VEGFR1, VEGFC, VEGFR2, Ang and Tie2 related to angiogenesis, raf and rasip1 related to ras/MAPK pathway activation, and cyclin D1 related to cell cycle were observed in the UR + TRG group. These results suggest that the vascular tumor promoting activity of TRG in rasH2 mice is extremely low in the present experimental condition. Further studies are necessary to clarify the carcinogenic susceptibility of PPAR γ agonists in rasH2 mice.

520 EVALUATION OF POSSIBLE CYTOTOXICITY AND MITOGENESIS IN HUMAN MICROVASCULAR ENDOTHELIAL CELLS TREATED WITH THE PPARγ AGONIST, TROGLITAZONE.

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Troglitazone is a thiazolidinedione that binds and activates the peroxisome proliferators-activated receptor γ (PPARγ). Previous studies demonstrated that the incidence of hemangiosarcomas in skin/subcutis, liver, bone marrow, spleen, adipose tissue, and heart was increased in mice treated with high doses of troglitazone (400 and 800 mg/kg) for 2 years. In addition, an increase in endothelial cell proliferation in the heart and brown fat tissue from mice treated with troglitazone for 1 and 2 weeks was observed. To investigate the direct mitogenic effects of troglitazone on endothelial cells, an immortalized human microvascular endothelial cell line HMEC1, was treated with different concentrations of troglitazone (10, 20, 25, 30, 40 and 50 μM) for 1, 3 and 6 days. The cell proliferation was measured by the MTT assay. Cytotoxicity was observed in a dose dependent manner from day 1 and the LC 50 on day 3 and day 6 was 18.7 and 20.2 μM, respectively. To evaluate the cell proliferative effects of troglitazone at low doses (0.001, 0.1, 1, 10 and 20 μM), HMEC1 cells, cultured without growth factors, were treated with troglitazone for 1, 3 and 6 days. No cell proliferative effects were observed. On the basis of these data, it appears that troglitazone has no direct mitogenic effects on human endothelial cells.

521 ROLE OF KUPFFER CELL IN WYETH-14, 643-INDUCED HEPATOCYTE PROLIFERATION IN NAÏVE C3H MICE.

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Kupffer cells (KC), the resident macrophages in the liver upon activation can produce an array of inflammatory and growth control mediators that can modulate acute and chronic responses of the liver including hepatic cancer. Wyeth-14,643 (Wyeth), a non genotoxic carcinogen and peroxisome proliferator has been previously shown to induce hepatocyte proliferation in rats via mechanisms involving KC. The objective of the present study was to investigate the role of KC in cell proliferation induced by Wyeth in naïve C3H mice, a strain commonly used in carcinogenicity studies. Two different experimental strategies were used; depletion of KC using liposome encapsulated clodronate (Lip-cld, 0.1 ml/10 g, iv) and inactivation of KC activity using glycine diet (5% glycine). LPS, a known activator of KC was used as a positive control (1 mg/kg, ip, 2X/week). Two time points (7 d and 21 d) were studied. Mini osmotic pumps containing BrdU (20 mg/ ml in saline) were surgically implanted subcutaneously to mice 7 d prior to killing the mice to facilitate measurement of DNA synthesis. Lip-cld treatment resulted in depletion of 99% of KC in the liver as confirmed by quantification of F4/80 positive cells. Wyeth treatment significantly increased the relative liver weight and DNA synthesis after 7 and 21 d treatment. LPS also induced hepatic DNA synthesis. Following KC depletion, the induction of DNA synthesis by LPS (after 7 and 21 days) and Wyeth (after 21 days) were significantly decreased. Glycine treatment did not reduce the number of F4/80 positive cells and similarly did not reduce LPS or Wyeth induced cell proliferation. In conclusion, our data indicates that Kupffer cells appear to participate in cell proliferation induced by exposure to Wyeth in C3H mice. (Supported in part by NIH CA100908).

522 GENE EXPRESSION PROFILES OF PERFLUOROOCTANOIC, -NONANOIC AND -DECANOIC ACIDS AND 8:2 FLUOROTELOMER ALCOHOL IN RAINBOW TROUT, A MODEL FOR HUMAN HEPATOCARCINOGENESIS.

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Previous studies have shown that perfluorooctanoic acid (PFOA) and structurally related perfluoroalkyl carboxylic acids (PFCAs) are estrogenic in rainbow trout, an animal model that represents the lack of sensitivity to peroxisome proliferators observed in humans. In this study, a toxicogenomics approach was used to examine the mechanism of action of PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA) and 8:2 fluorotelomer alcohol (8:2FtOH) in comparison to 17β-estradiol (E2) and the peroxisome proliferator, clofibrate (CLOF). A custom rainbow trout oligo DNA microarray was utilized to identify transcriptional profiles resulting from dietary exposure to each test chemical. E2, PFOA, PFNA and PFDA regulated many transcripts in common, including genes associated with vitellogenesis, regulation of transcription (*e.g.*, estrogen receptors 1 and 2), signal transduction (*e.g.*, NFκB1, IKKα) and immune response (*e.g.*, VHSV4, tcpbp). Although hierarchical clustering and Pearson correlation analyses showed that the expression profiles for E2 and the PFCAs tested were highly similar ($r > 0.7$), a number of genes were altered only in animals exposed to PFCAs. Notably, transcripts involved in the complement pathway (C3-3, C3-4, factor Bf-1, factor H and pentraxin) and the coagulation cascade (α2 macroglobulin, antithrombin, factor XII and plasminogen) were repressed in animals exposed to PFOA, PFNA and PFDA. 8:2FtOH increased expression of only a few estrogen-responsive genes (*e.g.*, vitellogenin, esr1); also, this chemical uniquely induced genes involved in oxidative stress response (*e.g.*, glutathione peroxidase, glutathione S-transferase). Few genes were responsive to CLOF treatment, which was poorly correlated with E2, 8:2FtOH, PFOA, PFNA and PFDA ($r < 0.3$). Studies are in progress to evaluate the tumor promoting potential of these perfluorinated compounds in trout. Supported by NIH grants ES07060, ES03850 and ES00210.

523 MECHANISMS OF INHIBITION OF GAP JUNCTION INTERCELLULAR COMMUNICATION THROUGH DIFFERENT MOLECULAR PATHWAYS.

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Gap junction intercellular communication (GJIC) maintains tissue homeostasis and the chronic closure of gap junctions has been associated with different pathologies, such as cancer. Regardless, the molecular mechanisms that are known to regulate GJIC are not fully understood. Currently, two pathways have been implicated in the regulation of GJIC; one dependent on Mek, and the second on the phosphatidyl choline specific phospholipase C (PC-PLC). We used a scrape-load dye-transfer assay to assess GJIC in a rat liver epithelial cell line. Firstly, cells were pretreated with either a specific inhibitor of Mek or PC-PLC to screen which pathway the selected growth factors and tumor promoters regulate GJIC. Then we determined the effect of the antioxidant, resveratrol, on these same factors to determine if resveratrol worked in a signaling specific or general antioxidant dependent way. TPA, EGF, TRAP-6 and lindane regulated GJIC through a Mek-dependent mechanism, whereas, low molecular weight PAHs, PCBs, dicumylperoxide, pentachlorophenol and perfluorodecanoic acid inhibited GJIC through PC-PLC. Resveratrol was an effective protector of GJIC inhibition caused by all compounds working through PC-PLC and Mek, but did not protect against compounds, such as perfluorooctane sulfonic acid, lauricidin and B-glycyrrhetinic acid, that did not regulate GJIC through either Mek or PC-PLC. These results indicate that promoters and protection from potential anticarcinogens is not necessarily global but mechanism-specific. Support: NIEHS grant #R01 ES013268-01A2 to BLU.

524 MECHANISM OF GENOMIC HYPOMETHYLATION DURING HEPATOCARCINOGENESIS INDUCED BY PEROXISOME PROLIFERATORS IN RATS.

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Genomic hypomethylation is a consistent finding in human tumors. Accumulated evidence underlines the role of DNA hypomethylation as one of the main events in tumorigenesis. Additionally, it has been suggested that DNA hypomethylation,

may be a sensitive biomarker for the carcinogenicity testing. Several possible mechanisms, such as alterations in metabolism of cellular methyl donors, disturbances in activity and/or expression of DNA methyltransferases (DNMTs), and presence of DNA lesions could contribute to loss of cytosine methylation during carcinogenesis. In the present study, we examined the role of these pathway disturbances in the mechanism of DNA hypomethylation during hepatocarcinogenesis induced by 4-chloro-6-(2,3-xylidino)-pyrimidynylthioacetic acid (WY-14,643) and di-(2-ethylhexyl) phthalate (DEHP), agents that belong to a class of chemicals called peroxisome proliferators. Male Fisher 344 rats were fed 0.1% WY-14,643, 1.2% DEHP, or NIH-07 control diet for 5 months. In the liver of rats exposed to WY-14,643, the level of genomic hypomethylation increased by ~2-fold, while the DEHP group hypomethylation increased by only 20%, as compared to age-matched control rats. Global DNA hypomethylation in livers from WY-14,643 group was accompanied by the accumulation of DNA lesions, profound cell proliferation, and diminished DNMT1 expression, while the status of methyl group metabolism was not compromised. In contrast, none of these parameters changed in livers of rats fed DEHP. Since WY-14,643 is much more potent carcinogen than DEHP, we conclude that a) the level of DNA hypomethylation is inversely related to the carcinogenic potential of toxicant, and b) accumulation of DNA lesions accompanied by increased cell proliferation and altered DNA methyltransferase expression may be a major driving factor leading to genomic hypomethylation during tumorigenesis.

525 ROLE OF C-MYC IN NICKEL-INDUCED HISTONE MODIFICATION ALTERATIONS.

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Nickel is a well known carcinogen, but the mechanism by which it causes cancer is still unclear. Nickel compounds are weakly mutagenic, but cause epigenetic gene silencing in cells. Our study demonstrated nickel compounds can increase H3 K9 dimethylation, H3 K4, K9 and K36 trimethylation levels. C-myc plays important roles in cells since almost 15% of the genes, which function in cellular proliferation, growth, apoptosis, protein synthesis, DNA replication, and angiogenesis, are regulated by this transcription factor. C-myc is believed to be involved in tumorigenesis in diverse human cancers. Here we show that nickel upreguated c-myc mRNA expression and protein levels in normal human lung cells (Beas-2B), which may contribute to nickel-induced cell transformation. However, we found that c-myc mRNA expression and protein levels were decreased in human lung carcinoma cells (A549) by nickel exposure. And it seems this difference was not iron- or oxygen-dependent, because our study shows that desferoxamine (DFO) and dimethyloxaloyglycine (DMOG) downregulated c-myc mRNA expression and protein levels in both cell lines. In fact global epigenetic effects can be seen by down-regulation or up-regulation of c-myc (Knoepfler et al., 2006.Wu et al., 2007), we also described that c-myc inhibitors (10054-F4 and U0126) increased H3 K9 dimethylation and H3 K36 trimethylation. Further studies will focus on the mechanism of nickel-induced c-myc alteration, and identifying the role of c-myc in these nickel-induced epigenetic alterations of histone modifications. (This work was supported by grant numbers 5R01ES014454-02 and 2R01ES005512-16A2, ES05512, ES00260, ES10344, and T32ES07324 from the National Institute of Environmental Health Sciences, CA16087 from the National Cancer Institute)

526 PHENOBARBITAL (PB) ALTERS THE METHYLATION STATUS OF KEY GENES DURING EARLY PHASES OF TUMORIGENESIS.

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Previously, only a few specific genes have been implicated in mouse liver tumorigenesis (MLT) (e.g., H-ras, Raf, Myc and β-Catenin), typically identified late in the process. DNA methylation, an epigenetic modification, plays multiple roles in tumorigenesis; hypomethylation (HypoMe) can activate oncogenes and hypermethylation can silence tumor suppressors. Methylation sensitive restriction digestion, arbitrarily primed PCR and capillary electrophoresis were used to detect unique regions of altered methylation (RAMs) that formed upon treatment with a tumor-promoting dose of PB for 2 or 4 wk, in B6C3F1 mice, sensitive to liver tumorigenesis, as compared to resistant C57BL/6 mice (Bachman et al., 2006). In this study, sequences representing unique RAMs in the B6C3F1 mice were cloned and annotated by BLAT searches. An informatics approach elucidated cellular functions/interactions of the gene products. Our hypothesis is that this approach can discern "new" genes involved in PB-induced tumorigenesis due to aberrant methylation. Of the 170 unique B6C3F1 RAMs that formed at 2 or 4 wk of PB promotion, 90 were cloned and 49 genes identified. Genes involved in invasiveness and metastasis are HypoMe at 4 wk: Tcf4, Ralgds and Tgfbr2 which facilitate epithelial-mesenchymal cell transition and Tnk2 plus Cma1. Rxrb, a binding partner of CAR, and Smrt/Ncor2, a corepressor of nuclear receptors (e.g., CAR), are newly methylated at 4 wk. Common protein targets/regulators of these genes were identifed to discern biological pathways that might be disregulated by altered methylation; HypoMe of Cma1 and Tcf4 may upregulate Vegf. We have annotated genes, which have not been previously linked to MLT, whose methylation statuses are altered uniquely in liver-tumor sensitive mice. By 4 wk, PB induces HypoMe of key genes involved in metastasis, a characteristic of late stage tumorigenesis. This is consistent with the idea that during early phases of tumorigenesis, cumulative epigenetic modifications can facilitate the clonal expansion of preneoplastic cells.

527 CHRONIC EXPOSURE TO HIGH-FAT DIET CAUSES RENAL PATHOLOGY IN NON-DIABETIC RATS.

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Evidence from animal and epidemiologic studies suggests that obesity may be one of the risk factors in renal cancer. To elucidate the role of high-fat diet induced obesity in the development of renal cancer, male Sprague-Dawley rats were fed a customized high-fat diet or standard chow for 11 months. Subsequent to exposure, life-history traits, plasma parameters, renal pathology, α2U-immunohistochemistry (IHC) and cell proliferation (PCNA-IHC) demonstrated: - The previously described rat subpopulation sensitive to high-fat diet exposure (Diet-Induced Obesity Sensitive, DIOsens), which developed a morbidly obese phenotype: body weights >1kg, and large epididymal, visceral and inguinal fat depots. A second, more high-fat diet-resistant subpopulation (DIOres), with modestly increased body weight (average 600g) and local fat depot sizes more reminiscent of chow-fed control rats. - High fat-diet exposure led to extensive mononuclear cell infiltration, protein casts, interstitial fibrosis and regenerative cell proliferation, as well as to the presence of pre-neoplastic lesions (atypical tubules). Control rats presented with marginal pathology. - PCNA-IHC corroborated the increased regenerative cell proliferation observed in DIOsens and DIOres rats, and absence thereof in control rats. - α2U accumulation was found in all groups tested, independent of the diet and severity of obesity. In conclusion, chronic high-fat diet leads to severe renal pathology, independent of the level of adiposity. While recurring cycles of tissue damage, inflammation and regeneration are critical for tumor promotion and -progression, it is speculated that chronic high-fat diet may propagate the survival and progression of spontaneous mutations and pre-neoplastic lesions. Above findings suggest a previously unknown phenomenon of dietary lipid induced renal toxicity, which may be highly relevant for the ongoing obesity epidemic.

528 ABERRANT EXPRESSION OF KEY REGULATORS OF MITOSIS AND CHROMOSOME SEGREGATION IN RAT KIDNEY FOLLOWING EXPOSURE TO OCHRATOXIN A.

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Ochratoxin A (OTA) is a potent renal carcinogen, but little is known regarding the mechanism of OTA carcinogenicity. Early histopathological alterations induced by OTA, which include single cell death, increased cell turnover and prominent nuclear enlargement, suggest that aberrant mitosis may be the principal cause of cell death and subsequent trigger for cell proliferation to compensate for cell loss. To gain further insight into the molecular mechanism of OTA toxicity, we used custom made qRT-PCR arrays to investigate expression of genes involved in cell cycle control and mitosis in kidneys of male F344 rats treated with 0, 21, 70 and 210 μg/kg bw OTA. Treatment with OTA at doses of 70 and 210 μg/kg bw for 90 days resulted in overexpression of several genes implicated in progression of mitosis and chromosome segregation, including Polo-like kinase 1, Aurora kinase B, cyclin-dependent-kinase 1 (Cdk1), cyclin B, cyclin E, cyclin A2, p21WAF1/CIP1, stratifin, topoisomerase II and Bub1b. Immunohistochemical analysis used to confirm results obtained by qRT-PCR also demonstrated increased expression of Cdk1, p21WAF1/CIP1, topoisomerase II, and increased phosphorylation of histone H3, a target of Aurora kinase B, in S3 proximal tubule cells, the target site of OTA toxicity. No effects were evident at the low dose or in the liver, which is not a target for OTA carcinogenicity. In the high dose group, alterations in gene expression occurred within 2 weeks of treatment and, therefore, before the onset of cell proliferation, suggesting that overexpression of mitotic regulators is an early event and not a consequence of enhanced cell turnover. Results from this study support our hypothesis that disruption of mitosis, resulting in blocked or asymmetric cell division, accompanied by an increased risk of aneuploidy acquisition, may play a critical role in OTA carcinogenicity and point to several key regulators of mitosis and chromosome segregation as potential mediators of these effects.
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529 PROPYLENE OXIDE: BIOMARKERS OF EXPOSURE, NASAL CELL PROLIFERATION, AND CANCER MODE-OF-ACTION IN RATS AND MICE EXPOSED *VIA* INHALATION.

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Propylene oxide (PO) reacts with cellular macromolecules such as DNA, hemoglobin (Hb), and glutathione (GSH). Chronic inhalation exposure to high levels of PO induced nasal tumors in rats and mice. Mode-of-action (MOA) studies have shown that, while the DNA adduct N7-hydroxypropylguanine (N7HPG) increases linearly with dose, the key event of induction of cell proliferation in nasal respiratory epithelium (NRE) has a non-linear dose-response. This study further characterized the MOA dose-response in F344 rats and studied these effects in B6C3F1 mice. Animals were exposed to PO via whole body inhalation (0, 50, 100, 200, or 400 ppm; 6 h/d; 5 d/wk; 4 wk), and selected tissues prepared for GSH, DNA, and Hb isolation and analysis. BrdU mini-pumps were implanted for the last 3 d of exposure. Nasal mucosa was examined microscopically to assess epithelial morphology and cell proliferation, and quantitative analysis of N7HPG and N-terminal Hb hydroxypropyl valine (HPVal) adducts, and apurinic (AP) sites was conducted. HPVal and N7HPG were detected in control animals, and increased linearly with PO exposure. Adduct levels were equivalent across species at each dose, indicating similar internal/target doses of PO, while AP site levels were the same for treated and control tissues. NRE from both species had a significant increase in target NRE cell proliferation at 400 ppm PO (BrdU incorporation), with lesser increases (non-significant in rat nasoturbinate target) at 200 ppm, but not at lower concentrations. Although adduct biomarkers increased linearly, a threshold dose-response was demonstrated for the key event, induction of cell proliferation in target NRE, with response beginning at 200 ppm PO, and corresponding with the tumor NOEL of 200 ppm. Thus these data support a threshold MOA cancer risk assessment for PO. Funded by ACC PO/PG Panel and Cefic PO & Glycols Sector Group.

530 CONSTITUTIVE ACTIVATION AND TARGETED DISRUPTION OF SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 3 (STAT3) IN MOUSE EPIDERMIS REVEAL ITS CRITICAL ROLE IN UVB-INDUCED SKIN CARCINOGENESIS.

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Recent studies from our laboratory have shown that Stat3-mediated regulation of gene expression is required for all three stages of skin tumor development induced by chemical carcinogenesis regimens, implying its critical role in chemically induced skin cancer. In current studies, the potential role of Stat3 in UVB-induced skin carcinogenesis was examined using skin-specific gain and loss of function transgenic mice, BK5.Stat3C and K5Cre.Stat3$^{fl/fl}$ mice. Constitutive activation of Stat3 in mouse epidermis significantly increased cell proliferation and epidermal thickening in response to UVB irradiation. The epidermis of Stat3-deficient mice was highly sensitive to UVB-induced apoptosis, whereas the epidermis of BK5.Stat3C mice was more resistant to UVB-induced apoptosis, compared to wild-types. Consistently, expression of target genes regulated by Stat3, such as cyclin D1 and Bcl-xL, was increased by Stat3 activation after UVB irradiation. Based on these results, two transgenic mouse models of Stat3 were subjected to a UV skin carcinogenesis regimen to examine the potential role of Stat3 in UVB-induced skin carcinogenesis. BK5.Stat3C mice were more sensitive to UVB irradiation than wild-type controls as evidenced by differences in skin morphology after 10 weeks of UVB irradiation. After 30 weeks of UVB irradiation, the formation of papillomas in BK5.Stat3C mice was greatly accelerated compared to wild-type mice and the percentage of BK5.Stat3C mice that developed papillomas was significantly greater than wild-type controls. In contrast, Stat3-deficient mice were more resistant to UVB irradiation than wild-type controls with reduced papilloma formation. These results suggest that Stat3 plays an important role in UVB-induced tumor formation and growth by increasing cell proliferation and survival. In addition, these data suggest that Stat3 is an excellent target for prevention of UVB-induced skin cancer.

531 USE OF MECHANISTIC DATA IN ASSESSMENT OF CARCINOGENICITY OF IPROVALICARB.

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Iprovalicarb (IPRO), a fungicide against a variety of pathogens, has been found to be non genotoxic. IPRO is rapidly absorbed orally with rapid excretion, and without accumulation in any tissue. Oncogenicity (diet) studies were performed in B6C3F1 male (M) and female (F) mice (M: 58.5, 283.4, or 1566.8 mg/kg/day; F: 97.4, 503.1, 2544 mg/kg/day) and Wistar rats (M: 26, 262.5, 1109.6 mg/kg/day; F: 31.7, 326.3, 1379.7 mg/kg/day). Thus, M mice received 29% more IPRO than M rats, F mice 46% more than F rats, and high dose (HD) F rats 20% more than M rats. Review of both studies by U.S. EPA concluded that IPRO was not carcinogenic to M and F mice (with adequate exposure and survival), but in M and F rats it was carcinogenic in 4 tissues; namely, in HD M rats, 3 (6%) malignant (MAL) osteosarcomas ($p<0.05$ pairwise); in HD F, 2 (4%) MAL Müllerian (MM) uterine tumors, in mid dose- MD-1 or 2% MM tumor, both not significant; in HD F, 2 (4%) benign (BE) urothelial cell (UC) papillomas; and in HD F, 3 (6%) combined BE and MAL follicular cell (FC) tumors of the thyroid ($p<0.05$ trend). In the MD F, 2 (4.2%) FC tumors ($p<0.05$ trend) were also present. The FC tumors were not significant. Thus, of the 4 tumor types, only the osteosarcomas were significant (pairwise) in M. In F, the 3 (or even 2) tumor types were not present together in any one of the 10 tumor-bearing rats, as might be expected of a compound related effect. IPRO was negative for DNA reactivity using 32P-postlabeling analysis in uterine and UCs. To examine whether there was any other IPRO effect that might underlie a nongenotoxic tumorigenic effect, the rate of proliferation was evaluated immunohistochemically by PCNA at 12 (interim sacrifice) and 24 months. At both intervals there was absence of a consistent dose related proliferative trophic effect in any of these 4 tissues. Thus, there is no causal relationship between IPRO exposure and neoplasia.

532 MODE OF ACTION FOR THE SYNTHETIC PYRETHROID METOFLUTHRIN-INDUCED RAT LIVER TUMORS: CONSTITUTIVE ANDROSTANE RECEPTOR (CAR) ACTIVATION AND ASSOCIATED LIVER CHANGES.

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Two-year treatment with high doses (900 and 1800 ppm) of metofluthrin produced hepatocellular tumors in both sexes of Wistar rats. To understand the mode of action (MOA) by which the tumors are produced, a series of studies examined the effects of metofluthrin on hepatic microsomal cytochrome P450 (CYP) content, hepatocellular proliferation, hepatic gap junctionnal intercellular communication (GJIC), oxidative stress and apoptosis conducted after one or two weeks of metofluthrin treatment in rats. Metofluthrin induced CYP2B, resulted in increased liver weights which were associated with centrilobular hepatocyte hypertrophy, and induction of increased hepatocellular DNA replications. Metofluthrin also suppressed hepatic GJIC in vitro and induced oxidative stress and increased antioxidant enzymes, but no alteration in apoptosis. The above parameters related to the key events in metofluthrin-induced liver tumors were observed at or below tumorigenic dose levels. All of these effects were reversible upon cessation of treatment. Metofluthrin did not cause cytotoxicity or peroxisome proliferation. These data may suggest that the MOA of metofluthrin-induced rat liver tumor involves the effects of liver CYP induction via CAR activation and subsequent stimulation of increased hepatocellular proliferation. The global gene expression profile strongly supports the postulated MOA. Based on analysis with the ILSI/IPCS MOA frameworks, including comparison with the results of another chemical, phenobarbital, acting by a similar MOA, it is reasonable to conclude that metofluthrin will not have any hepatocarcinogenic activity in humans at expected levels of exposure.

533 INTERACTIONS BETWEEN ARYL HYDROCARBON RECEPTOR AND ESTROGEN RECEPTOR ALPHA: ROLE OF AF2 DOMAIN.

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Aryl hydrocarbon receptor (AHR) and estrogen receptor α (ERα) are ligand activated transcription factors that mediate the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and estrogens respectively. Reciprocal cross-talk between AHR and ERα has been described in a number of different systems, and has been implicated to play a role in various human diseases such as breast cancer and endocrine disruption. Our laboratory has recently reported ERα recruitment to AHR target genes, CYP1A1 and CYP1B1 in response to TCDD treatment. These data prompted us to characterize the ERα domains necessary for recruitment to AHR target genes. We have created ERα mutants of the activation function 1 (AF1) (ERα-ΔAF1), AF2 (ERα-AF2) and DNA-binding (DBD) (ERα-DBD) domains and transiently transfected mutants as well as wildtype ERα into ERα-negative HuH-7 human liver hepatoma cells. Using chromatin immunoprecipitation (ChIP) analysis followed by quantitative real-time PCR (Q-PCR) we observed recruitment of transfected wildtype ERα to CYP1A1 and CYP1B1 when cells were treated with AHR

agonists TCDD and 3-methylcholantrene (3MC) alone or in combination with estrogen treatment. However, recruitment of ERα-ΔAF1 was comparable to wildtype ERα. ERα-AF2 recruitment to CYP1A1 and CYP1B1 was lower compared wildtype ERα. Western blot analysis has revealed reduced ERα-AF2 recruitment to CYP1A1 and CYP1B1 was not due to differences in protein expression of mutant ERα. These results indicate ERα interacts with AHR and is recruited to AHR-target genes in response to AHR agonist treatment through the AF2 domain and not through the AF1 as previously reported. These data also suggest that additional co-regulators may be involved in ERα recruitment to AHR target genes. Collectively, our findings provide new insight into the complex cross-talk between AHR and ER signaling pathways.

534 CHIP-ON-CHIP ANALYSIS OF TCDD-DEPENDENT ARYL HYDROCARBON RECEPTOR BINDING TO HUMAN PROMOTER TILING ARRAYS REVEALS AHR RECRUITMENT TO ESTROGEN RECEPTOR ALPHA PROMOTER AND ENHANCER REGIONS.

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In this study, chromatin immunoprecipitation followed by microarray analysis (ChIP-on-chip) of chromatin isolated from TCDD treated T-47D human breast cancer cells was used to determine the direct genomic targets of AHR using a human promoter array from Affymetrix. Asynchronous T-47D cells were treated for 1 h with TCDD, chromatin was isolated, ChIP assays performed using antibodies directed against AHR. Immunoprecipitated DNA was linearly amplified and hybridized to the human promoter tiling 1.0R arrays that contain 25,500 promoter regions. Using XRAYBiotique software and the MAT (microarray analysis tool) algorithm with a bandwidth of 300, maxgap of 300, min run of 150 and a significance level of $p<0.00001$ we observed 1641 significant promoter regions. Using this statistical cut-off CYP1A1 and CYP1B1 were among the identified promoter regions validating our methodology. Interestingly, the estrogen receptor α promoter (ESR1) was also among the identified promoter regions. Conventional ChIP analysis confirmed TCDD- and 3MC-dependent recruitment of AHR to ESR1 promoter and enhancer regions. ERα was also present at these genomic loci in an AHR-ligand independent manner. These findings were also supported by Genomatrix ChIPInspector software. Pathway analysis revealed that many of the gene products from the other identified target promoters were involved in ESR1 regulation. These data identify AHR as an important regulator of ESR1 uncovering new insight into the functional overlap between AHR and ERα signalling.

535 EFFECT OF ENVIRONMENTAL ENDOCRINE DISRUPTORS ON 3-METHYLCHOLANTHRENE-DEPENDENT INDUCTION OF ARYL HYDROCARBON RECEPTOR ACTIVITY AND RECRUITMENT OF ESTROGEN RECEPTOR ALPHA TO AHR TARGET GENES.

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Our previous studies have shown that AHR ligands induce recruitment of ERα to AHR target promoters and that this recruitment was increased by estrogen cotreatment. In this study we investigated the effect of the environmental estrogens, 4-t-octophenol, Bisophenol A, and Tamoxifen and estrogen on 3MC-dependent AHR activity and promoter occupancy of ERα in T-47D human breast cancer cells. We also compared the effects of serum (FBS) vs dextran coated charcoal treated serum (DCC-FBS) responses on AHR activity and ERα promoter occupancy were also examined. Time course ChIP analysis of treated T-47D human breast cancer cell line showed that 3MC induced robust binding of AHR and ERα to CYP1A1 or CYP1B1 enhancers after 0.5 h of treatment, recruitment was reduced after 2h before rebounding after 4 h exposure. Cells were than treated for 1, 4 or 24 h with 3MC, alone or in combination with the abovementioned endocrine disruptors. Both BPA and 4-t-octylphenol induced a similar level of promoter occupancy of AHR and ERα to CYP1A1 and CYP1B1; however, when cells were grown in DCC-FBS cotreatment of 3MC+Tamoxifen resulted in significantly lower promoter occupancy levels of both AHR and ERα at CYP1A1 and CYP1B1 compared to 3MC alone. No differences were observed when cells were grown in complete FBS containing medium. Despite the apparent reduced recruitment of AHR to CYP1A1 or CYP1B1 no difference the mRNA expression levels of either gene were observed. Interestingly, ERα and AHR were recruited to the estrogen responsive ESR1 and GREB1 promoter and enhancer regions in a 3MC- or TCDD-dependent manner. Collectively, these data further support the strong functional overlap between AHR and ERα signalling pathways.

536 PHYTOESTROGENS REGULATE PS2 AND PROGESTERONE RECEPTOR (PGR) GENE EXPRESSION AT THE CHROMATIN LEVEL THROUGH ESTROGEN RECEPTOR IN MCF-7 CELLS.

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The estrogenic activity associated with phytoestrogens (PE), is believed to be responsible for the regulation of pS2 and PgR genes through the estrogen responsive element (ERE) present in the 5'-flanking region. The gene regulation by PE is thought to involve a direct DNA-protein interaction. In addition the PgR promoter contains an activator protein 1 (AP1) response element which may play a role in mediating the estrogenic activity of PE through an indirect DNA-protein interaction. We have previously demonstrated that pS2 and PgR are upregulated at the transcriptional level using endpoint assays such as Northern blotting and QRT-PCR. However, there has been no report showing direct regulation of pS2 or PgR by PE at the chromatin level. Therefore, the objective of this study is to demonstrate if the regulation of pS2 and PgR genes by PE occurs at the chromatin level. We demonstrated the dose dependant association of PE/ER complex to the promoter of pS2 and PgR genes by inducing MCF-7 cells with increasing concentrations of PE genistein (GE) or coumestrol (CO) in the presence and absence of 1μM ICI 182,780 (ICI) for 24hrs. We also demonstrated the time dependant association by inducing MCF-7 cells with 100nM GE or CO for 0, 12, 24 and 48hrs. The DNA/protein complex formed as a result of PE induction was analyzed by ChIP assay, western blot analysis and QRT-PCR. The immunoprecipitation of PE/ER complex with ER-α (ER-712) or ER-β showed an association of ER with the promoter DNA of both pS2 and PgR genes. PCR analysis confirmed the binding of PE/ER complex to the ERE and AP1 binding site, in the promoter DNA of these genes. Taken together, our data determined that PE regulates pS2 and PgR gene expression by interacting with ER-α and their responsive elements present in the promoter region.

537 ARYL HYDROCARBON RECEPTOR (AHR) ACTIVATION DECREASES PROLIFERATION BUT DOES NOT INCREASE APOPTOSIS IN MAMMARY EPITHELIAL CELLS.

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Exposure to the AhR agonist 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) during pregnancy causes severe defects in mammary gland differentiation, including stunted longitudinal growth, decreased ductal branching, poor formation of alveolar structures, suppressed expression of milk proteins, and failure to nutritionally support offspring. However, the mechanism by which AhR activation alters mammary differentiation remains unclear. Alterations in proliferation, differentiation, or early apoptosis of the epithelial cells can lead to failed lactogenesis. Recent studies suggest that AhR is involved in molecular cascades that regulate cell cycle progression and apoptosis. The present study was conducted to determine the effects of AhR activation on mammary epithelial cell proliferation and apoptosis. Impregnated mice were treated orally with TCDD or peanut oil vehicle control, and mammary tissue was collected on day of pregnancy 6, 9, 12 or 17. Treatment with TCDD did not increase caspase-3 activity or the number of apoptotic cells in mammary glands. In contrast, there was a 50% reduction in the number of BrdU-positive mammary epithelial cells in glands from TCDD-treated animals, and this was observed as early as the 6th day of pregnancy. AhR alters the level of the cell cycle regulatory protein p21 in other tissues. However, proliferation during pregnancy did not correlate with detectable changes in p21 levels in mammary glands. In addition, development of glands in p21-deficient (p21-KO) mice was analyzed and scored according to ductal branching, longitudinal and lateral growth patterns, and lobulo-alveolar structures. AhR activation impaired mammary differentiation similarly in pregnant p21-KO and wild-type mice. These results suggest that AhR activation impairs mammary differentiation during pregnancy through cell cycle arrest rather than increasing apoptosis, and this effect appears to be p21-independent.

538 OVEREXPRESSION OF ARNT DOES NOT ALTER CROSSTALK BETWEEN THE ARYL HYDROCARBON RECEPTOR AND HYPOXIA PATHWAYS IN PLHC-1 CELLS.

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Hypoxic events are increasing in frequency and severity due to human activity. We have shown previously that hypoxia exacerbates the embryotoxic effects of polycyclic aromatic hydrocarbons (PAHs) in killifish (Fundulus heteroclitus) and zebrafish (Danio rerio). It has been proposed that the mechanism underlying the in-

teractions between hypoxia and PAHs is competition for a dimerization partner (ARNT) shared by the hypoxia pathway and the AhR pathway. Several studies addressing AhR/hypoxia crosstalk in mammalian cell lines have produced contradictory results regarding whether reciprocal crosstalk actually occurs between these pathways and the role ARNT plays in this interaction. Using a topminnow (Poeciliopsis lucida) hepatocarcinoma cell line (PLHC-1), we assessed hypoxia-AhR crosstalk in fish with and without overexpression of ARNT. PLHC-1 cells were transfected using a two-way factorial design with either an AhR or hypoxia luciferase reporter vector in the presence or absence of an ARNT expression vector. All cells were co-transfected with a Renilla control vector. Cells were exposed to normoxia (21% O2) or hypoxia (1% O2) and various concentrations of the PAH benzo[a]pyrene (BaP) for 24 hours, then assayed for luciferase activity. Reciprocal crosstalk was observed. Hypoxic exposure reduced induction of the AhR reporter by BaP and BaP exposure decreased induction of the hypoxia reporter by hypoxic exposure. Overexpression of ARNT caused an increase in activity of both the AhR and the hypoxia reporter, however the pattern of reciprocal crosstalk remained. These studies suggest that while there is reciprocal crosstalk between the AhR and hypoxia pathways, competition for ARNT does not explain the occurrence of this crosstalk. Research funded by NIEHS Duke Superfund Basic Research Center (P42 ES10356), NIEHS-supported Integrated Toxicology and Environmental Health Program (T32 ES07031), and EPA STAR Fellowship to C. Fleming.

539 INTEGRATED REGULATION OF CYTOCHROME P450 1A1 mRNA EXPRESSION BY HYPOXIA AND AHR SIGNALING PATHWAYS IN HUMAN PULMONARY MICROVASCULAR ENDOTHELIAL CELLS.

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The aryl hydrocarbon receptor (AHR) and hypoxia-inducible factors (HIFs) are members of the basic helix-loop-helix/PER-ARNT-SIM family of DNA-binding proteins that mediate organismal responses to xenobiotics and hypoxia, respectively. Since studies have suggested that these pathways may interact in their regulation of cytochrome P450 1A1 (CYP1A1) mRNA expression, we assessed endogenous CYP1A1 mRNA expression when human pulmonary microvasculature endothelial cells were simultaneously exposed to hypoxia and either 1.0 nM 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) or AHR siRNA for 18 hrs. In contrast to prior studies, simultaneous AHR activation and hypoxia exhibited both inhibitory and stimulatory crosstalk on CYP1A1 mRNA expression. Severe hypoxia (1% O2) was inhibitory, while modest hypoxia (2.5% O2) was stimulatory. Basal CYP1A1 mRNA expression was suppressed independently by both AHR siRNA and hypoxia, while the combination of the two treatments exhibited less than additive inhibition at both levels of oxygen. In addition, AHR mRNA expression was suppressed by severe hypoxia (1% O2) and this suppression was restored to basal levels by HIF-2alpha siRNA. Interestingly, suppression of CYP1A1 mRNA expression by hypoxia was attenuated by HIF-2alpha siRNA, but expression was not fully restored back to levels observed under normoxia. These data suggest that suppression of CYP1A1 mRNA expression by hypoxia is HIF-2alpha-dependent, in part, and may result from suppression of AHR expression. Future studies will investigate the degree to which hypoxia and HIF-2alpha regulated AHR and CYP1A1 protein expression. This work was supported by NIH HL078914 and AHA 0550028Z to MKW.

540 REGULATION OF PEROXISOME PROLIFERATOR ACTIVATED RECEPTORS (PPARS) BY E6-ASSOCIATED PROTEIN (E6AP).

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PPARs (PPARα, PPARβ, and PPARγ) are nuclear hormone receptors that regulate genes involved in lipid and lipoprotein metabolism, glucose homeostasis, inflammation, and cell growth. Regulation of these pathways is crucial to controlling diseases such as diabetes, atherosclerosis, cancer, and chronic inflammation. Upon ligand binding, PPARs bind to the promoters of target genes, and multiple PPAR-protein interactions with coregulators and heat shock proteins have been identified that regulate gene expression. The ubiquitin-proteasome system, a major regulator of nuclear receptor dynamics has also been shown to affect PPAR activity by modulating ligand-induced PPAR levels. This study examines the role of E3 ubiquitin ligase E6AP on PPARs. Previously shown to regulate the progesterone and the estrogen receptors, E6AP was seen in this study to decrease the basal levels of PPARα, PPARβ, and PPARγ. Overexpression of E6AP decreased PPAR transactivation, as observed using a PPAR-response element driven luciferase reporter construct. Identifying the mechanism involved and particularly the role of the ubiquitin-ligase activity of E6AP will improve our understanding of the connections between the ubiquitin-proteasome system and PPARs. This can provide insights into the action of existing PPAR-based therapeutics and aid in developing more effective treatment strategies.

541 COMPARISON OF THE ACTIVITIES OF CARBOXYLATES AND SULFONATES OF PERFLUOROALKYL ACID (PFAA) OF VARIOUS CARBON CHAIN LENGTHS ON MOUSE AND HUMAN PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-ALPHA (PPARα) IN COS-1 CELLS.

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PFAA are used in consumer products and persist in the environment. They elicit adverse effects on rodent development and neonatal survival, and may act via PPARα to produce some of their effects. The induction of mouse and human PPARα activity by perfluorinated carboxylic acids (PFCAs) and perfluorinated sulfonic acids (PFSAs) of various carbon chain lengths was tested using a transiently transfected COS-1 cell assay. COS-1 cells were transfected with either a mouse or human PPARα receptor luciferase reporter plasmid. After 24 hours, cells were exposed to either vehicle control (DMSO [0.1 %]), PPARα agonist (WY14643, [10 μM]), PFAA vehicle control (water or DMSO [0.1 %]); perfluorooctanoic acid (PFOA) or perfluorononanoic acid (PFNA) at 0.5-100 μM; perfluorobutanoic acid (PFBA), perfluorodecanoic acid (PFDA), or perfluorohexanoic acid (PFHxA) at 5-100 μM; perfluorohexane sulfonate (PFHxS) at 5-100 μM; or perfluorobutane sulfonate (PFBS) or perfluorooctane sulfonate (PFOS) at 1-250 μM. After 24 hrs of exposure, cells were lysed and PPARα plasmid luciferase activity was measured. Lowest observed effects concentration (LOEC) was determined by ANOVA ($p < 0.05$). Each of the PFAAs activated the mouse and the human PPARα plasmid in a concentration dependent fashion, except PFDA, which did not activate human PPARα plasmid at any concentration. Activation of both mouse and human PPARα was positively correlated with carbon chain length, for PFBA (C4), PFHxA (C6), and PFOA (C8). PFDA (C10) induced high activity in mouse PPARα (LOEC =5 μM). PFOA produced a biphasic response with a plateau at high doses. PFSAs generally induced lower activity of mouse and human PPARα compared to PFCAs. We have found that PFCAs of different chain lengths induce activity of the mouse and human PPARα differently, and that PFSAs are weaker activators of mouse and human PPARα than PFCAs. This abstract does not necessarily reflect EPA policy.

542 SPECIES DIFFERENCE IN THE HEPATIC RESPONSE TO PERFLUOROCTANOIC ACID (PFOA).

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Previous studies have demonstrated that PFOA can activate peroxisome proliferator-activated receptor-α (PPARα). The present study tested the hypothesis that there is a species difference in PFOA-induced liver effects using PPARα humanized transgenic (PPARα-hTg) mice. Wild-type, PPARα-null and PPARα-hTg were treated with either 0.0, 0.1, 0.3 or 1.0 mg/kg of PFOA or 500 mg/kg of clofibrate via oral gavage for up to 14 days After 24 hours, significant changes in gene expression associated with increased lipid catabolism and activation of PPARα were observed in clofibrate-treated wild-type mice and PPARα-hTg mice, but not in PPARα-null mice. These acute changes were not found in mice treated with PFOA. Interestingly, serum levels of PFOA were significantly lower after 14 days of treatment in the Sv/129 mice used for this study as compared to CD1 mice used in other studies. After 14 days of administration, a PPARα-dependent increase in liver weight was observed in PFOA-treated wild-type mice at the higher doses, but did not occur in similarly treated PPARα-null or PPARα-hTg mice. A PPARα-dependent increase in mRNAs encoding enzymes involved in fatty acid catabolism was detected by microarray analysis and confirmed by qPCR in wild-type and PPARα-hTg mice treated with PFOA. Increased expression of CYP3A4 and CYP2B10 were also observed in PFOA-treated mouse liver from all genotypes. Results from the present studies demonstrate that PFOA can modulate similar changes in gene expression required to facilitate fatty acid catabolism via activation of both the mouse and human PPARα. In contrast, the mild hepatomegaly induced by PFOA is differentially modulated by mouse versus human PPARα. Additionally, results from these studies suggest that other receptors including CAR and PXR may also be important in modulating liver-specific effects resulting from PFOA exposure.

543 ROLE OF DISCOIDIN DOMAIN RECEPTOR 1 ON NITRIC OXIDE-INDUCED APOPTOSIS IN MACROPHAGES.

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Discoidin domain receptor 1 (DDR1), nonintegrin collagen receptor, is a receptor tyrosine kinase (RTK) with a unique extracellular domain homologous to discoidin 1 of Dictyostelium discoideum. DDR1 is known to play a role in cell attachment, migration, survival, and proliferation. However, little is known about the molecular mechanisms underlying the role of DDR1. A recent paper has reported that p53 induction and activation of DDR1 kinase counteract p53-mediated apoptosis and influence p53 regulation through a positive feedback loop. This study was designed to determine the role of DDR1 in nitric oxide (NO) -induced apoptosis. NO plays important roles in physiologic processes including nerve system and inflammation. NO generation initiates apoptotic cell death in different experimental system. In our study, the administration of S-nitroso-N-acetyl-DL-penicillamine (SNAP), a NO donor, decreased the cell viability and induced apoptotic cell death in J774A.1 macrophages. Interestingly, the expression of DDR1 was decreased by treatment of SNAP. On the contrary, the inhibition of expression of DDR1 by siRNA increased cell viability and reduced NO-induced apoptosis. These results demonstrate that DDR1 has anti-apoptotic effect against NO-induced apoptotic cell death in macrophages by regulating the signaling molecules. We suggest that modulation of DDR1 signaling in macrophages may be useful in controlling apoptosis signal transduction.
This work was supported by Brain Korea 21.

544 IDENTIFICATION OF BORIC ACID-RESPONSIVIE RYANODINE RECEPTOR ISOFORMS IN TUMOR AND NON-TUMOR PROSTATE CELL LINES.

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Boric acid is a dietary component found in fruits and vegetables and is found naturally in the environment in soil and water and as an agricultural pollutant. Low levels reduce the incidence and mortality of prostate cancer. Boric acid has been shown to inhibit intracellular calcium signaling mediated through the NAD/cADPR calcium signaling pathway in DU145 prostate cancer cells over a dose range from chemopreventative to toxic. Our current studies have also shown that low doses of boric acid can inhibit calcium release from the ryanodine receptor (RyR) in the endoplasmic reticulum in response to RyR agonists in DU145 cells. The dose of boric acid required to inhibit ryanodine receptor-dependent release of stored calcium is 10 to 100 fold higher in non-tumor PWR1E cells than DU145 prostate cancer cells, indicating that there may be differences in RyR isoforms or expression levels between cell lines. In this project we have tested the hypothesis that boric acid requires specific RyR isoforms for its effect on calcium signaling. Our objective is to identify the specific RyR isoforms that potentially interact with boric acid in LNCaP and DU145 prostate cancer cell lines versus PWR1E non-tumor prostate cells. The identification of different isoforms of the receptor was assessed with restriction enzyme digestion of reverse-transcribed mRNA from all three cell lines. Overall RyR mRNA expression in DU145 and LNCaP cells treated with varying doses of boric acid was measured using reverse-transcriptase and real-time PCR. There was no significant change in mRNA expression levels in cells treated with 0, 1, 10, 50, and 250 μM boric acid indicating that the interaction is at the receptor and not the transcription level. Funded by UCTSR&TP and US Department of Defense.

545 REACTIVE CYSTEINES C1040 AND C1303 OF RYANODINE RECEPTOR TYPE 1 INFLUENCE RESPONSES TO CELLULAR OXIDATIVE STRESS AND GLUTATHIONE DEPLETION.

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Redox modulation of the skeletal muscle ryanodine receptor type 1 (RyR1) plays a key role in determining the responsiveness of the Ca^{2+} release channel to physiological modulation. The sensitivity of RyR1 to glutathione (GSH) may be conferred by previously identified hyper-reactive cysteines, including positions C1040 and C1303. Wild type RyR1 (WT), C1040S, and C1303S mutations were stably expressed in HEK-293 cells and their sensitivity to agents modulating RyR1 was determined with the MTS assay. 1, 4 naphthoquinone (NQ) decreased cell viability in a dose-dependent manner, but WT cells were significantly less sensitive than null cells lacking RyR1 expression. WT and C1303S cells were significantly less susceptible to NQ compared to C1040S expressing cells. Activator of RyR1, 4-chloro-*m*-cresol (4CMC) induced a dose-dependent increase in cell viability in the null, WT and C1303S cells, whereas it inhibited growth of C1040S expressing cells. GSH depletion was induced by inhibiting γ-glutamyl cysteine synthase with buthionine sulfoximine (BSO) for 24 hr, but depletion by itself did not impair cell viability for any of the genotypes tested. Challenge of null and WT cells following depletion of GSH revealed no difference in sensitivity to NQ while both C1040S and C1303S mutants showed reduced sensitivity to the cytotoxic effects of NQ. Depletion of GSH attenuated 4CMC-enhanced cell viability in WT and C1303S cells. These results indicate a protective role of RyR1 in NQ mediated cytotoxicity and 4CMC-enhanced cell growth. The report also suggests an involvement of hype-reactive cysteines C1040 and C1303 in determining responses to oxidative stress. Supported by NIH PO1 ES 11269 (INP), RO1 AR43140 (PDA and INP) and AR018687 (GM).

546 COMPARISON OF METHAMPHETAMINE, COCAINE, AND APOMORPHINE-INDUCED BEHAVIORAL SENSITIZATION IN MU-OPIOID RECEPTOR KNOCKOUT MICE.

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Repeated administration of psychostimulant to rodents may result in a progressively enhanced and persistent behavioral response which is called "behavioral sensitization", a model for investigation of drug addiction. Mechanisms responsible for psychostimulant-mediated sensitization remain unclear, although the CNS mesolimbic dopamine system is thought to be played an important role. The present study investigated the role of mu-opioid receptor in development of behavioral sensitization to psychostimulants methamphetamine, cocaine, and apomorphine using mu-opioid receptor knockout mice. To initiate sensitization, mice were given a single daily intraperitoneal injection of saline or a psychostimulant (methamphetamine 0.6 mg/kg; cocaine 10 mg/kg; apomorphine 2 mg/kg) for 7 consecutive days. On day 11 (after 4 abstinent days) mice were challenged with one injection of saline or a psychostimulant (the same as initiation). Locomotor activities were monitored 30 min before and 120 min after drug injection on experimental days 1, 7 (to evaluate the development of sensitization), and 11 (to evaluate the expression of sensitization). Result indicated that repeated administration of methamphetamine and cocaine led to the development and expression of behavioral sensitization in wild-type mice but not in mu-opioid receptor knockout mice. Contrary, the mu-opioid receptor knockout mice were more sensitive than their wild-type controls to apomorphine-induced behavioral sensitization. Both methamphetamine and cocaine are indirect dopamine antagonists whereas apomorphine is a direct dopamine antagonist. The results might suggest that mu-opioid receptor modulates the development of psychostimulant sensitization and dopamine receptors are implicated in this modulation (Supported by NIH grant RR-P20 RR1701).

547 GABAA AND PERIPHERAL BENZODIAZEPINE RECEPTOR LIGANDS ACTIVATE LIVER NUCLEAR RECEPTORS.

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The constitutive androstane receptor (CAR) is expressed almost exclusively in hepatocytes, resides in the cell cytoplasm in an inactivate state and is activated by many chemicals with which it does not directly interact. An indirect (non-ligand) activator of CAR is the sedative phenobarbital (PB), which causes CAR to translocate to the cell nucleus. Besides PB, multiple neuroactive chemicals including other barbiturates, benzodiazepines and steroids are transcriptional activators of CAR-target gene CYP2B6 in the liver and in cultured primary hepatocytes, but this response is not conserved in human hepatoma cell line HuH7. Interestingly, a large percentage of hepatic CYP2B6-inducing compounds are well-characterized ligands of the gamma amino butyric acid A receptor subtype (GABAA), a gene family that includes at least sixteen central nervous system-enriched inhibitory neurotransmitter receptors. Reverse transcription-polymerase chain reaction (RT-PCR) assays and western blot analyses of GABAA receptor mRNA and protein levels revealed that GABAA receptors are differentially expressed in HuH7 cells versus primary human hepatocytes. A chemical library of GABAA receptor ligands was screened in primary human hepatocytes for activation of CAR target CYP2B6 and pregnane x receptor (PXR) target CYP3A4. Similarly, HuH7 cell line was transiently transfected with CAR, a ligand-inducible splice variant of CAR (CAR3) or PXR expression plasmids in order to measure CAR- and PXR-driven luciferase reporter activity in

response to treatments from the chemical library. Results show multiple ligands of GABAA receptors and peripheral benzodiazepine receptor (PBR) are direct (ligand) and indirect (non-ligand) activators of CAR, CAR3 or PXR. Hepatic GABAA receptors or the PBR may be causally involved in indirect (non-ligand) activation of CAR by neuroactive pharmacological agents.

548 UNIQUE TRANSCRIPTION START SITES AND DISTINCT PROMOTER REGIONS DIFFERENTIATE THE PREGNANE X RECEPTOR (PXR) ISOFORMS PXR 1 AND PXR 2.

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The pregnane X receptor (PXR) is known as the xenosensing receptor responsible for coordinated regulation of metabolic genes in response to diverse xenobiotic challenges. In particular, the ability of PXR to regulate CYP3A4, the enzyme capable of metabolizing more than 60% of all pharmaceuticals, defines its metabolic importance. Currently the list of PXR ligands and target genes is extensive, yet investigations into the regulation and expression of PXR are few. After an initial review of available sequence data, we discovered discrepancies in the 5'UTR and TSS characterizations of the human PXR gene, and subsequently endeavored to define TSSs and proximal promoters for isoforms PXR 1 and PXR 2. RT-PCR and primer extension experiments performed on RNA from human liver identified two TSSs for each receptor isoform. These results extended the 5'UTR sequence of each isoform and defined new proximal promoters for both. Candidate response elements for liver-enriched transcription factors and other nuclear receptors were found in both proximal promoters. Quantitative PCR from human liver illustrated a highly variable expression profile for total PXR; yet PXR 2 expression while modest represented a consistant 2-5% of total PXR expression, despite the observed variability. Transfection experiments demonstrated comparable ability of PXR 1 and PXR 2 to transcriptionally activate the CYP3A4 promoter. Collectively, comparable function, consistant expression, and independent regulation suggest that PXR 2 is capable of contributing to the cumulative function of PXR and should be included in the larger investigations of PXR expression and regulation.

549 THE CIRCADIAN RHYTHM OF ENTEROHEPATIC CIRCULATION OF BILE ACIDS IN MICE.

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Bile acids (BAs) are amphipathic cholesterol metabolites synthesized in liver, secreted into intestine via bile to aid in the absorption of lipophilic nutrients. The majority of BAs are reabsorbed by ileum and returned to the liver by the portal vein. The purpose of this study was to reveal the circadian expression pattern of genes involved in the enterohepatic circulation (EHC) of bile acids. Liver and ileum were collected from mice at 2 am, 6 am, 10 am, 2 pm, 6 pm, 10 pm and mRNA was quantified. Mice are nocturnal animals, thus a number of mRNAs of BA synthesis enzymes and transporters increase between 6 pm-10 pm, indicating the existence of a circadian network. These transcripts include the BA synthesis enzymes (cholesterol 7α-hydroxylase [Cyp7a1] and sterol 12α-hydroxylase [Cyp8b1]) in liver, the intestinal BA uptake transporters (apical sodium dependent bile acid transporter [Asbt] and organic anion transporting polypeptide 2b1 [Oatp2b1]), and the intestinal basolateral BA efflux transporter organic solute transporter α (Ostα). Interestingly, the master nuclear receptor for BAs, farnesoid X receptor (FXR), is also highest expressed in the evening in both liver and ileum. Nuclear receptors pregnane X receptor (PXR), hepatocyte nuclear factor 1α (HNF1α), HNF4α, liver X receptor (LXR) and liver receptor homolog-1 (LRH-1) exhibit similar circadian expression profiles. These nuclear receptors may coordinately mediate the time course of BA EHC. The circadian transcription factors periods (PERs), cryptochromes (CRYs), as well as the PARbZip (proline and acidic amino acid-rich basic leucine zipper) transcription factors are also highly transcribed during the evening in mouse liver, underlying the regulation of peripheral clocks by the central circadian oscillator. In conclusion, there is a circadian rhythm of a number of enzymes and transporters involved in the EHC of bile acids, which is likely mediated by multiple transcription factors. (Supported by R01ES09649, R01ES09716, RR021940.)

550 TRANSCRIPTIONAL REGULATION OF PREGNANE X RECEPTOR BY PROTEIN ARGININE METHYLTRANSFERASES.

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Histone modification is an important mechanism for the epigenetic regulation of gene expression. Protein arginine methyltransferase 1 (PRMT1), which methylates arginine 3 of histone H4 (H4R3), has recently been shown to regulate the nuclear receptor transcriptional activity. Pregnane X receptor (PXR), an orphan nuclear receptor, regulates the expression of a set of genes involved in phase I, II xenobiotic/drug metabolism as well as transporters. Many of them have been implicated in various diseases and drug-drug interactions. One of the major targets of PXR in human is the cytochrome P450 3A4 (cyp3a4), which is responsible for the first pass biotransformation of over 50% clinical drugs. Based on coimmunoprecipitation/histone methyltransferase (HMT) assay with Flag-tagged human PXR, we found HMT(s) associated with PXR in human hepatoma (HepG2) cells that specifically methylated H4R3 as determined by the N-terminal microsequencing, suggesting PRMT1 is the major methylating enzyme. Through GST-pulldown, Co-IP/Western, mammalian two hybrid assays, we found that PRMT1 interacts with PXR directly. Using chromatin immunoprecipitation assay, we detected a ligand dependent recruitment of PRMT1 to cyp3a4 regulatory regions with concomitant methylation of H4R3. PXR transcriptional activity was abolished in the PRMT1 null mice ES cells. Suppression of PRMT1 activity by the inhibitor ADOX or knock down of PRMT1 by RNAi abolished the rifampicin induction of PXR-regulated cyp3a4. Our results suggest that PRMT1 plays an important role in the PXR-mediated gene expression. Its methyltransferase activity is essential for this function (Supported in part by NIEHS Grant ES09859, ES09106 and American Heart Association Grant 0355131Y).

551 CROSSTALK BETWEEN THE AHR AND CAR PATHWAYS IN HEPATOCYTES.

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The aryl hydrocarbon receptor (AhR) and the constitutive androstane receptor (CAR) are cytosolic transcription factors that regulate the expression of a battery of drug-metabolizing enzymes including members of the CYP1A and the CYP2B/2C/3A subfamilies, respectively. Both receptors are known to be sequestered in the cytosol and upon xenobiotic activation translocate to the nucleus to modulate gene expression. Using cultures of human and mouse primary hepatocytes, we have examined the crosstalk between these distinct receptor pathways via high throughput nuclease protection assays of mRNA content, enzymatic activities to assess functional protein, and nuclear translocation assays. We found the AhR ligand b-Napthoflavone (BNF) to induce AhR target genes mRNA for CYP1A1 and CYP1A2 up to 160- and 58-fold, respectively, in primary human hepatocytes. Interestingly, BNF also induced the CAR target gene CYP2B6 mRNA up to 26-fold in these hepatocyte cultures. In addition, the CAR selective activator 6-(4-chlorophenyl)imidazo[2,1-b]thiazole-5?carbaldehyde O-(3,4-dichlorbenzyl)oxime (CITCO) was observed to induce CYP2B6 up to 43-fold and CYP1A1 mRNA by 10-fold. To further explore this apparent crosstalk between AhR and CAR receptor pathways, we inhibited CAR-mediated induction via the selective mouse CAR antagonist androstenol in the presence and absence of prototypical AHR and CAR inducers. CYP450 enzymatic activity and mRNA content were modulated in response to these stimuli. Finally, the effect of BNF on the expression and translocation of CAR was examined in a dose-dependent manner to determine if this compound causes induction of CAR expression and/or affects the nuclear accumulation of human CAR compared with known CAR activators (phenobarbital and CITCO). Overall these data indicate that crosstalk between these distinct transcription factors is evident, and further investigation of the details molecular mechanisms of this crosstalk is warranted.

552 CHARACTERIZATION OF AH RECEPTOR AND ARNT PROTEIN EXPRESSION IN YEAST.

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The aryl hydrocarbon receptor (AHR) and the aryl hydrocarbon receptor nuclear transporter (ARNT) are well characterized bHLH-PAS transcription factors shown to regulate expression of xenobiotic metabolism genes in fish, bird, rat, mouse, and human tissues and cell lines in a ligand-dependent fashion. While significant evaluation of the receptor signaling pathway has been carried out in higher eukaryotes, few groups have characterized AHR and ARNT using the yeast Saccharomyces cerevisiae, one of the simplest eukaryotes. Previously, labs have utilized yeast strains expressing exogenous AHR and ARNT in order to elucidate the function of evolutionarily conserved molecular chaperones and co-chaperones that constitute the latent Ah receptor complex, but these studies did not evaluate expression and interaction of AHR and ARNT at the protein level. The goal of the current study was to produce yeast strains in which the dynamics of AHR and ARNT protein turnover could be evaluated. To this end, mouse AHRb-2 and ARNT cDNAs were stably integrated independently into the yeast genome directly downstream of a galactose-inducible promoter. These strains were then mated in order to produce strains expressing both AHR and ARNT with or without a dioxin-responsive reporter. The galactose promoters were induced with the addition of galactose to the growth

medium followed by total cellular protein isolation. Western blotting revealed a band at approximately 104kDa and 85kDa when stained with anti-AHR and anti-ARNT antibodies, respectively. Dioxin treatment resulted in ligand-dependent reporter activity in the expressor strain carrying the pLXRE5-Z beta-galactosidase reporter plasmid. To further confirm that AHR and ARNT form functional heterodimers and bind DNA in yeast, electrophoretic mobility shift assays and immunoprecipitations were performed. The ability to detect functional AHR and ARNT in the various yeast strains at the protein level suggests that these lines will be appropriate for future studies of AHR and ARNT turnover. Supported by ES ES015481.

553 INTER-SPECIES AND –ISOFORM DIFFERENCES IN FUNCTIONS OF TWO ARYL HYDROCARBON RECEPTORS (AHR1 AND AHR2) FROM AVIAN SPECIES.

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The aryl hydrocarbon receptor (AHR) is a ligand activated intracellular protein that is responsible for toxic effects of dioxins and its related compounds (DRCs). Our previous study demonstrated that avian species possesses two distinct AHR isoforms (AHR1 and AHR2). To understand the functional diversities of two AHRs from avian species, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) binding affinities and in vitro DRC-induced transcriptional activities of two AHR isoforms from chicken (Gallus gallus), black-footed albatross (Phoebastria nigripes), common cormorant (Phalacrocorax carbo) and jungle crow (Corvus macrorhynchos) were investigated. AHR1 and AHR2 isoforms from all the four avian species exhibited specific binding to [3H]TCDD, as assessed by velocity sedimentation. An in vitro reporter gene transactivation assay revealed that AHR2s from chicken, albatross and cormorant had reduced transcriptional efficiencies as compared to AHR1s from the corresponding species. Particularly, chicken AHR2 exhibited dramatically reduced transactivation. In contrast, the transcriptional ability of crow AHR2 was almost comparable to that of AHR1. One possible reason accounting for this difference in transactivation may be the structural difference in C-terminal halves . Furthermore, tissue expression profiles of AHR isoform mRNAs in cormorant and crow revealed that AHR1s were expressed in most tissues, whereas AHR2s were detectable in limited tissues. However, comparison of hepatic mRNA expression levels of two AHR isoforms showed a species difference; AHR1 is dominant than AHR2 in cormorant, in contrast to crow, in which AHR2 is the major form. These results suggest that function of AHR2 mediating the response to DRC exposure is diverse even within avian species, and this fact raise questions on the use of chicken as a conventional avian model for studying the toxicology of DRCs.

554 UNIQUE STRUCTURAL DETERMINANTS CONFER LOW TCDD RESPONSIVENESS TO AN ARYL HYDROCARBON RECEPTOR FROM THE FROG *XENOPUS LAEVIS.*

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Frogs are remarkably insensitive to the toxic effects of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD). Previous studies in *Xenopus laevis* (African clawed frog) revealed that aryl hydrocarbon receptors (AHRs) from this species bind TCDD with low affinity and exhibit low responsiveness in reporter gene assays. In other vertebrates, including mice, humans, and birds, differences in TCDD toxicity and AHR binding affinity result from differences in specific amino acids within the ligand binding domain (LBD). In this study, we sought to identify structural determinants within *X. laevis* AHR1β associated with low TCDD responsiveness in luciferase reporter gene assays. Substitution of the entire LBD with the corresponding sequence from the AHR^{b-1} mouse restored TCDD responsiveness characteristic of the intact mouse receptor; thus, residues within the LBD are likely responsible for the functional differences. Surprisingly, the frog AHR contains all residues previously associated with high affinity TCDD binding in mice and birds, suggesting a role for other amino acids. Alignment of the amino acid sequences of high-affinity LBDs with frog AHR1β revealed two residues that were unique to the *X. laevis* receptor but well conserved in the other species. These were mutated to the corresponding mouse residue (T256A and S263P), but in reporter gene assays, each change produced only a very modest increase in TCDD responsiveness, neither approaching the nearly 50-fold difference in EC50 ordinarily observed between AHR^{b-1} and frog AHR1β. Simultaneous mutation of both residues produced a less than additive increase. Changing additional frog-specific residues to the mouse sequence, including S294Q and S371T, had no effect. These results suggest that different, less phylogenetically conserved residues or specific combinations of residues underlie the low TCDD responsiveness of the frog AHR. Ongoing studies will examine additional mutant constructs in order to identify the unique structural determinants of TCDD affinity of *X. laevis* AHRs. [NIH R15 ES011130]

555 DIFFERENTIAL FACTORS AFFECTING BINDING OF SILICA AND TITANIUM DIOXIDE (TIO_2) PARTICLES TO THE SCAVENGER RECEPTOR CYSTEINE-RICH (SRCR) DOMAIN OF MACROPHAGE ASSOCIATED RECEPTOR WITH COLLAGENOUS DOMAIN (MARCO).

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It is well known that silica can cause chronic lung inflammation leading to silicosis, whereas TiO_2 is relatively benign. We hypothesize that the differential outcome may relate to the binding of these particles to scavenger receptors on the macrophages. The scavenger receptor, MARCO is a 210-kDa trimeric membrane protein belonging to the SRCR domain super family of proteins. These proteins have a variable number of SRCR domains in their extracellular regions. Furthermore, the MARCO ligands are polyanionic in nature. The arginine "RXR" motif in the SRCR domain of MARCO was recently reported to be required for bacterial binding. Substantial evidence has accumulated that MARCO also binds unopsonized environmental particles like silica and TiO_2. However, the exact binding site and the factors affecting the particle-receptor interaction are unknown. Studies in our laboratory using Chinese Hamster Ovary (CHO) cells expressing different SRCR mutants of MARCO have identified for the first time that unlike bacteria, the entire SRCR domain appears to be required for the binding of silica and TiO_2. Apoptosis assays in these cells confirmed a required role of the SRCR domain in silica-mediated cytotoxicity. Paradoxically, inspite of avidly binding to MARCO, TiO_2 did not induce apoptosis in the transfected cells. To further understand this contrasting observation, the divalent cations calcium and magnesium were shown to be necessary for TiO_2 binding, whereas silica binding did not require divalent cations. Collectively, these studies suggest that the SRCR domain is required for silica binding and subsequent apoptosis. The dissimilar dependence on divalent cations by MARCO in binding silica and TiO_2, may serve as a basis for the difference in apoptotic outcome and development of silicosis.
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S 556 DRUG-INDUCED MITOCHONDRIAL TOXICITY – NOVEL INSIGHTS-NOVEL TOOLS.

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Mitochondria have been increasingly recognized as a target of drug toxicity resulting in disruption of bioenergetics and causing oxidant stress in sensitive organs including CNS, heart, and skeletal muscle. In addition, there is increasing awareness that mitochondria are key mediators of drug toxicity in a number of other organs including liver and kidney, often causing mitochondrial outer membrane permeabilization and release of cell death proteins. Furthermore, certain underlying disease states like diabetes, infections, or neurodegenerative diseases can greatly alter mitochondrial function and make the mitochondria more vulnerable to drug toxicity. Conventional in vitro approaches in drug discovery and development have often failed to detect mitochondrial dysfunction, and there are few animal models which would readily reveal mitochondrial liability. This symposium will focus on new approaches to detect, understand, and predict mitochondrial toxicity. These novel approaches include transcriptional fingerprinting of mitochondrial changes, transgenic mouse models, and novel in vitro tools.

S 557 TRANSCRIPTIONAL SIGNATURE OF MITOCHONDRIAL TOXICITIES.

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Mitochondrial function is obligatory to tissue function. Accordingly, chemical-induced interference with mitochondrial performance is associated with assorted metabolic sequalae indicative of bioenergetic failure. Conventional attempts to develop diagnostic indicators of mitochondrial dysfunction focused on characterizing a specific metabolic phenotype, with particular emphasis on high-energy phosphate metabolites. Though marginally successful, alterations in metabolic status is a non-

specific and late indicator of mitochondrial toxicity, often not manifest until well into the moribund state. The reason is that cells are equipped with various compensatory mechanisms designed to sustain bioenergetic status until the terminal stages of the disease process. This presentation will discuss the biological basis for shortcomings of metabolic indicators of mitochondrial dysfunction with special emphasis on the transcriptional regulation of intermediary metabolism that is essential to sustain cell function. Within this is a common transcriptional signature indicative of a compensatory switch to anaerobic metabolism to sustain a viable bioenergetic status in the face of chemical-induced mitochondrial toxicity.

S 558 MITOCHONDRIA IN DRUG-INDUCED LIVER INJURY (DILI).

U. A. Boelsterli. *Pharmaceutical Sciences, University of Connecticut, Storrs, CT.*

Mitochondria are not only a frequent subcellular target of many chemicals including drugs but they also play a pivotal role as mediators of the toxic response. For example, a vast variety of drugs have been implicated in disrupting mitochondrial function in hepatocytes. However, independently of compromised energy homeostasis, these drugs (or their metabolites) also trigger mitochondria-mediated signaling (e.g., through oxidant stress-mediated Trx2 oxidation, JNK1/2 activation, or tBid formation leading to Bax/Bak activation) that can result in mitochondrial permeability transition (mPT) and/or mitochondrial outer membrane permeabilization (MOMP) and lethal cell injury. Novel antioxidants and superoxide dismutase mimetics (e.g. Mito-CP) that are specifically targeted and delivered to mitochondria can greatly reduce the mitochondrial superoxide levels and fully protect cells from toxicity, underscoring the role of mitochondrial oxidant stress in causing drug-induced cell injury. Identifying a drug-associated mitochondriotoxic hazard alone, however, does not readily explain why liver injury associated with many drugs is often very rare in patients, while the majority of recipients tolerate these drugs well. We have recently developed the working hypothesis that underlying mitochondrial abnormalities may be a plausible determinant of susceptibility to idiosyncratic DILI. In fact, minor impairment of the mitochondrial antioxidant defense systems can sensitize hepatocytes to the prooxidant and proapoptotic effects of such drugs. A recently developed animal model (the heterozygous Sod2 knockout mouse) indicates that mild and clinically silent underlying mitochondrial stress can greatly potentiate drug-induced mitochondrial damage and unmask overt liver injury in vivo. Clinical and experimental data support the concept that cumulative damage to mitochondria, induced by mitochondria-targeting drugs, if superimposed on genetic or environmentally induced abnormalities in mitochondrial function, may trigger overt DILI in susceptible individuals.

S 559 INHIBITION OF ETHANOL EFFECTS BY MITOCHONDRIAL THIOREDOXIN-2.

J. Hansen. *School of Medicine, Emory University, Atlanta, GA.* Sponsor: Y. Will.

Ethanol consumption is associated with numerous pathologies, including the progression of fatty liver. Many mechanisms have been described to elucidate how ethanol relates to disease, of which one commonly supported mechanism associated with ethanol toxicity is through the induction of mitochondrial oxidative stress. Our work has focused on understanding the role of the mitochondrial specific antioxidant and potential oxidoreductase, thioredoxin-2 (Trx2). Isolated mitochondria overexpressing Trx2 produced less hydrogen peroxide, suggesting a possible protective role against oxidative stress originating in the mitochondria. In vitro studies have shown that Trx2 is oxidized by ethanol treatments and that Trx2 overexpression protects against ethanol-induced cell death. Utilizing Trx2 transgenic mice, Trx2 was protective against the development of chronic ethanol-induced fatty liver, alterations in liver glutathione redox potential and changes in extracellular cysteine redox potentials. These data support the mitochondria as a target of ethanol toxicity and importantly, show that Trx2 is an important mediator of ethanol-induced oxidative damage.

S 560 LINKING MITOCHONDRIAL DYSFUNCTION TO HYPERGLYCEMIA: IMPACT OF MITOCHONDRIAL FIDELITY AND OXIDATIVE STRESS ON DIABETES AND ITS COMPLICATIONS.

C. M. Palmeira. *IMAR, Mitochondrial Research Group, Department of Zoology, University of Coimbra, Coimbra, Portugal.*

Hyperglycemia resulting from uncontrolled glucose regulation is widely recognized as the causal link between diabetes and diabetic complications. Four major molecular mechanisms have been implicated in hyperglycemia-induced tissue damage: activation of protein kinase C (PKC) isoforms via de novo synthesis of the lipid second messenger diacylglycerol (DAG), increased hexosamine pathway flux, increased advanced glycation end product (AGE) formation, and increased polyol pathway flux. Hyperglycemia-induced overproduction of superoxide has been suggested as the causal link between high glucose and the pathways responsible for hyperglycemic damage. In fact, diabetes is typically accompanied by increased production of free radicals and/or impaired antioxidant defense capabilities, indicating a central contribution for reactive oxygen species (ROS) in the onset, progression, and pathological consequences of diabetes. Besides oxidative stress, a growing body of evidence has demonstrated a link between various disturbances in mitochondrial functioning and type 2 diabetes. A reduction in the efficiency of ATP production linked to decreased efficiency of mitochondrial oxidative phosphorylation is a feature of impaired glucose homeostasis. Mutations in mitochondrial DNA (mtDNA) and decrease in mtDNA copy number have also been linked to the pathogenesis of type 2 diabetes. The study of the relationship of mtDNA to type 2 diabetes has revealed the influence of the mitochondria on nuclear-encoded glucose transporters, glucose-stimulated insulin secretion, and nuclear-encoded uncoupling proteins (UCPs) in beta-cell glucose toxicity. Since a range of mitochondrial factors contribute to the pathogenesis of diabetes, the direct effects of hyperglycemia on mitochondrial function and the regulation of mitochondrial function at a transcriptional level in response to hyperglycemia, should be taken into account to enable the selective targeting of drugs designed to modulate the function of this organelle for therapeutic gain.

S 561 STRATEGIES TO REDUCE NCE ATTRITION DUE TO MITOCHONDRIAL TOXICITY-DESIGNING NOVEL SCREENING METHODS.

Y. Will. *Drug Safety Research and Development, Pfizer, San Diego, CA.*

Mitochondrial dysfunction is a common mechanism of drug-induced toxicity for a variety of therapeutics, such as certain antiviral drugs, lipid-lowering drugs, NSAIDs and certain cancer chemotherapeutics. Early identification of new chemical entities (NCE) that perturb mitochondrial function is therefore of significant importance in drug discovery if attrition due to toxicity is to be avoided. To date there are no high throughput assays (HTS) available to assess mitochondrial function. We have tested a variety of new technologies capable of measuring mitochondrial function in a rapid throughput format. These include oxygen sensors to measure mitochondrial respiration in isolated mitochondrial and cells, immunocapture of individual electron transport chain proteins that can identify inhibitors of mitochondrial electron transport, metabolic profiling using solid oxygen and pH measurements and immunohistochemsitry for in vivo applications. We discuss the strength and limitations of new HTS applicable screens and provide recommendations of where to position these assays within drug development

S 562 ENVIRONMENTAL INFLUENCE ON FEMALE PUBERTY AND BREAST TUMORIGENESIS.

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Breast cancer is a complex disease, resulting from both genetic and environmental influences. Environmental exposures during critical windows of mammary gland development may be responsible for altering tissue susceptibility resulting in increased risk of future breast cancer. The pubertal hypothesis suggests that exogenous agents such as endocrine disrupting chemicals or other dietary factors, may mimic estrogen or influence their levels, such that the period of rapid development of the mammary gland is extended, putting the gland at higher risk for a transition to carcinogenesis. The NIEHS and NCI established the Breast Cancer and the Environment Research Centers (BCERC) Network, a unique multidisciplinary, translational grant program that includes basic biology, epidemiology, and community outreach and translation components to address the pubertal hypothesis. A primary objective of the BCERC Network is to explore the influence of a select set of exposures and activities on the onset of breast development and progression through puberty, early onset of puberty being among the most reliable risk factors for future beast cancer. Research within the Network spans a spectrum of biological organization: molecular mechanisms controlling regulation of differentiation of gland stem cells; insights into genomic and proteomic pre-disposition for disease; the impact of environmental exposures (including diet and obesity) at sensitive windows of development on gland development and the future risk of developing breast cancer as well as a cohort study of puberty designed to look at both environmental and genetic factors, and when possible their interactions, evaluating the impact of environmental exposures (broadly defined to include dietary, endocrine disruptors, consumer product use and socioeconomic factors) on progression to and through puberty in young girls. The aim of this symposium is to present the latest findings relating early environmental exposures with alterations in pubertal milestones that predispose towards breast cancer.

S 563 THE EFFECT OF ENVIRONMENTAL EXPOSURES ON MAMMARY STEM CELLS DURING PUBERTY.

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Mammary epithelium has regenerative capacity consistent with the presence of adult stem cells (ASC), but there are currently no specific markers to identify mammary ASC in situ. Label-retaining cells (LRC), shown to contain ASC in other epithelia, can serve as surrogates to define the architecture of mammary stem/progenitor cell distribution. We developed novel computational microscopy and multi-scale analyses to characterize LRC in the mouse mammary gland. LRC proliferated during tissue development, were relatively quiescent at homeostasis in the adult gland and rarely co-localized with differentiation markers. By image analysis we identified a distinct nuclear morphology for LRC and a 3.5-fold enrichment in LRC in the large ducts proximal to the embryonic origin of the epithelium. To test whether the differential LRC distribution was indicative of the functional localization of ASC, mammary epithelial cells isolated from proximal versus distal tissue were sorted on the basis of the mammary ASC surface markers CD24 and CD49f. $CD24^{+}CD49f^{high}$ cells showed proximal enrichment that correlated with a 2.5-fold increase in the regenerative potential of proximal epithelium in transplantation assays. Multi-scale in situ imaging in conjunction with cell sorting and functional analysis suggests an ASC reservoir proximal to the embryonic origin of the mouse mammary gland. This methodological approach has now been extended to rat mammary gland and applied to mammary glands from rodents exposed to known breast carcinogens and dietary supplements to determine whether dysregulation of the mammary stem cell contributes to carcinogenesis. *This project was carried out as part of the NIEHS/NCI Breast Cancer and the Environment Research Center.*

S 564 PRENATAL EXPOSURE TO BISPHENOL A (BPA) INDUCES GENOMIC ALTERATIONS IN THE RAT MAMMARY GLAND.

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This study analyzes the genomic signature of the mammary gland of rats exposed prenatally to BPA. Pregnant Sprague Dawley rats received 25 or 250ug BPA/kg body weight (low and high dose groups) or equivalent volume of sesame oil (control group) from gestation day 10 through delivery. On days 21, 35, 50 and 100 of life, female litters from each group were sacrificed. 10 animals per group were used for RNA extraction for genomic signature screening using Agilent platform with 22,000 features. Statistical significance was determined based on a false discovery rate cut-off of 5% and fold change of 2 times. The genes were annotated according to known functions and canonical pathways. Based on the gene ontology the immune system and the immune lymphatic system development functions were over-represented as a consequence of BPA exposure. The pattern of expression varies according to the dose. At the low dose the maximum level of genes expression occurs at 35 and 50 days of age with 30 genes and 26 canonical pathways including IL-10, apoptosis and chemokines signaling being affected. Inversely the mammary gland of the animals at 21 and 100 days of age were the most significantly affected by high dose exposure to BPA. 87 genes related to the immune system were affected by the BPA exposure at 100 days of age. These genes affected 62 canonical pathways; among them the IL-2, 4, 6 10, antigen presentation, apoptosis signaling, B cell receptor, chemokines, death receptor signaling, leukocytes extravasation, natural killer cells, NFβκ signaling, T cell receptor, TGFβ and VEGF signaling. BPA exposure during the development of the litter affects the gene expression of the immune system and lymphatic development. The high dose BPA treatment has an effect in a larger number of genes and extends the period of these changes to include the entire lifespan of the animal. This may indicate a widening of the window of susceptibility to carcinogenesis. *This project was carried out as part of the NIEHS/NCI Breast Cancer and the Environment Research Centers.*

S 565 NEONATAL/PREPUBERTAL BISPHENOL A EXPOSURE ALTERS THE MAMMARY PROTEOME, DECREASES APOPTOSIS AND INCREASES SUSCEPTIBILITY FOR CHEMICALLY-INDUCED MAMMARY CANCER IN RATS.

C. Lamartiniere. *University of Alabama at Birmingham, Birmingham, AL.*

Exposure to environmental chemicals during critical periods of development can play an important role in determining potential for biochemical insult later in life. We hypothesize that exposure to estrogenically active chemicals during an active period of mammary gland development will alter the biochemical blue print in this tissue and susceptibility for mammary cancer in the adult. We have investigated the potential of the endocrine disruptor, bisphenol A (BPA), when given during the neonatal/prepubertal period to predispose for chemically-induced mammary cancer in rats. For our studies, lactating Sprague Dawley rats were treated with BPA on days 1-20 postpartum with the intention of exposing the offspring via the mother's milk to BPA. At day 21, the offspring were weaned. At day 50 postpartum, 30 female offspring from each treatment group were treated with 30 mg dimethylbenz(a)anthracene (DMBA)/kg BW. Tumor palpations revealed that rats exposed neonatally/prepubertally to vehicle, low- and high- dose BPA developed 2.84 ± 0.30, 3.82 ± 0.55 and 5.00 ± 0.42 tumors/animal, respectively. Using discovery proteomics we have found that neonatal/prepubertal BPA exposure results in differential regulation of several proteins in the mammary glands of prepubertal and adult rats, including GRP78 and RAD51, proteins associated with apoptosis and DNA repair, respectively. Immuno-blot analysis confirmed regulation of these two proteins. Neonatal/repubertal BPA exposure was also shown to significantly decrease apoptosis. These data demonstrate that early exposure to BPA can alter can alter the mammary proteome, decrease apoptosis and increase susceptibility for chemically-induced mammary cancer in rats. *This project was carried out as part of the NIEHS/NCI Breast Cancer and the Environment Research Centers.*

S 566 THE ENVIRONMENTAL IMPACT OF DIET AND OBESITY ON BREAST CARCINOGENESIS.

D. Clegg[1], R. Gear[1], J. Schneider[1], M. Mistry[1], K. Olson[2] and S. Haslam[2]. [1]*University of Cincinnati, Cincinnati, OH and* [2]*Michigan State University, East Lansing, MI.* Sponsor: E. Maull.

This presentation will focus on the role of dietary fatty acids and obesity and how they impact breast carcinogenesis. An overview of what is known about the impact of diet will be given, followed by a description of the research being conducted at both Michigan State University and the University of Cincinnati. Our data indicate that regardless of obesity, certain fatty acids appear to enhance carcinogenesis in two rodent models. High fat diets appear to change the morphology of the mammary gland altering TEB, TD, and growth into the surrounding fat pads. The mammary glands appear to be 'estrogen resistant', a feature we are beginning to believe occurs in the presence of high fat diets. Additional information will be presented about the role of fatty acids and maternal imprinting and the impact this has on female offspring's genetic profile. New findings suggest that diets high in oleic acid appear to be promotional with respect to carcinogenesis, whereas other diets high in safflower oil or saturated fatty acids are not promotional. Furthermore, data will be presented suggesting oleic acid binds to a GPCR, a novel GPR40, in the mammary gland, which then activates the insulin signaling and growth cascade facilitating carcinogenesis. Our data suggest that oleic acid activates the mTOR growth pathway and that blocking oleic acid activation of this pathway with Rapamycin decreases carcinogenesis susceptibility. These findings will have a significant impact on current nutritional recommendations. *This project was carried out as part of the NIEHS/NCI Breast Cancer and the Environment Research Centers.*

S 567 THE BREAST CANCER AND THE ENVIRONMENT RESEARCH CENTERS: A NOVEL TRANSDISCIPLINARY APPROACH TO UNDERSTANDING THE CAUSES AND MECHANISMS OF BREAST CANCER.

D. Winn[1], E. A. Maull[2], G. Collman[2], L. Reinlib[2] and S. Lynch[1]. [1]*National Institute of Environmental Health Sciences, Research Triangle Park, NC and* [2]*National Cancer Institute, Rockville, MD.*

The Breast Cancer and the Environment Research Centers were developed and organized into two research projects, epidemiology and animal research, and Community Outreach and Translation Cores (COTCs), that includes representatives from the advocacy community. Care was taken to ensure integration of epidemiology research into meaningful animal research efforts, incorporation of animal research efforts into epidemiologic settings, communication of animal and epidemiology research results to the public, and collaborations between advocates and scientists throughout the BCERC research agenda. A coordinated epidemiologic study is being conducted at three sites to investigate the determinants of puberty in girls, with specific attention focused on understanding the shift toward earlier puberty among adolescent girls, the identification of environmental exposures in young girls, and the interplay between genetic variations and environmental exposures that may put them at increased risk of future breast cancer. Finally, the Network, through its COTCs, will integrate the biological, toxicologic and epidemiologic data on mammary gland development throughout a woman's lifespan in order to produce public health messages on the role of specific environmental stressors in breast cancer development and how to reduce exposure to those agents. This presentation will discuss findings from the epidemiologic investigations and summarize how the biology and epidemiology projects inform each other. Examples of how the scientific results are translated for the general public will be included.

S 568 OXIDANT AIR POLLUTION AND CHILDHOOD ASTHMA.

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Ozone is one of the primary constituents of smog. Under the Clean Air Act, EPA has set protective health-based standards for ozone. However, in 2005, 98 million people lived in U.S. counties with ozone concentrations above the 8 hr National Ambient Air Quality Standard of 0.08. There continues to be concern about the long term sequelae of widespread ozone exposures in sensitive populations, particularly children. This concern has been heightened by a recent surge in asthma prevalence in children, with disproportionately higher morbidity and mortality due to asthma for inner city children. The lung continues to grow and differentiate into adulthood. Interaction with air pollutants, such as ozone, during postnatal development of the respiratory system may alter the normal pattern of either growth or differentiation in such a way as to predispose to disease. Recent research has defined several important features regarding ozone exposure in children including: 1) critical windows of susceptibility to ozone for both the structural and immune components of the respiratory tract, 2) genetic markers for increased risk for decrements in lung function due to ozone exposure and 3) interaction effects with allergic sensitization and preexisting inflammation. The purpose of this session is to bring together the most current basic and applied research on the influence of ozone on the development of the lung.

S 569 OXIDANT AIR POLLUTION AND CHILDHOOD ASTHMA: GENE, ENVIRONMENT AND GENE-ENVIRONMENT INTERACTION.

T. S. Islam and F. D. Gilliland. *Preventive medicine, University of Southern California, Los Angeles, CA.* Sponsor: L. Van Winkle.

Airways are continuously exposed to oxidants from exogenous sources including air pollution (especially ozone, nitrogen oxides and traffic exposure) that can lead to oxidative stress which is thought to play a central role in asthma pathogenesis. We hypothesized that these exogenous sources of oxidative stress would be associated with increased risk of asthma among children and would modify the association between different anti-oxidant genes (such as GSTP1 and HMOX1) and asthma. To test our hypothesis we utilized longitudinal data from 4,118 participants in the Children's Health Study who were asthma-free at enrollment. New cases of physician-diagnosed asthma, lung function and risk factors for asthma were assessed annually at school visits from 1993 to 2003. Genetic data was available for 67% of this initial cohort. Community air monitoring was performed in each study community during the study period (1993-2003). The CHS results support an increased risk of asthma across several determinants of oxidative stress. Playing multiple team sports, a marker for total dose of ozone exposure, was associated with increased risk of asthma among children who lived in high ozone communities (HR 3.3, 95%CI 1.5-5.8). Furthermore, participation in team sports also increased the risk of asthma among children who were genetically susceptible to oxidative stress due to presence of Ile homozygotes of Ile105Val polymorphism of GSTP1 (HR 6.15, 95%CI 2.2-7.4). We also observed that, HMOX1 'short' alleles (< 23 repeats), which upregulates heme oxygenase activity, were associated with reduced risk for new onset asthma among non-Hispanic whites (HR 0.64, 95% CI 0.41-0.99) and this protective effect was largest in children residing in low-ozone communities (HR 0.48, 95%CI 0.25-0.91) (interaction p-value=0.003). We conclude ambient pollutants such as ozone is an important risk factor of asthma in genetically susceptible children.

S 570 GENETIC MECHANISMS OF SUSCEPTIBILITY TO OZONE-INDUCED LUNG INJURY: NEW INSIGHTS.

S. R. Kleeberger. *NIEHS, Research Triangle Park, NC.* Sponsor: L. Van Winkle.

Numerous epidemiological investigations have associated exposures to environmental oxidants with increased morbidity and mortality in urban areas throughout the U.S. and other countries. Inter-individual variation in lung responses to oxidant pollutants in otherwise healthy individuals, and association of candidate gene polymorphisms with responses to oxidants in clinical studies, suggests genetic background is an important determinant of pollutant susceptibility. We have utilized traditional and in silico positional cloning approaches in mice to identify genes that determine differential responsiveness to ozone-induced injury and inflammation, including Tnf (tumor necrosis factor alpha) and Tlr4 (toll-like receptor 4). Recent investigations have also identified MHC Class II and Marco as additional novel candidate genes. Proof-of-concept investigations have validated the importance of these genes in the mouse. Studies are ongoing to determine whether functional polymorphisms in orthologous genes associate with ozone susceptibility in humans.

S 571 EFFECTS OF OXIDANT EXPOSURE ON IMMUNE SYSTEM DEVELOPMENT IN EARLY LIFE.

L. A. Miller. *University of California, Davis, Davis, CA.* Sponsor: L. Van Winkle.

Chronic air pollutant exposure can exacerbate the clinical symptoms of asthma and has been proposed to contribute to the development of allergic airways disease. The immune mechanisms that initiate asthma during childhood are not well understood and the adjuvant properties of air pollutants have not been defined. It is known that the postnatal period of development is a highly dynamic phase for the pulmonary mucosal immune system, making this an important "window of susceptibility" for immunomodulation by inhaled pollutants and allergens. As such, developmental maturity of the immune and structural cell constituents of the lung will affect the overall response to environmental exposures and may initiate the early stages of allergic airways disease. The primary objective of this presentation is to discuss the effects of oxidant exposure on early pulmonary immune mechanisms and explore pathways for immunomodulation in the context of a non-human primate model of childhood asthma.

S 572 CRITICAL TIMEPOINTS FOR POSTNATAL DEVELOPMENT OF INFLAMMATORY AND EPITHELIAL DEFENSE MECHANISMS: OZONE AND LPS EXPOSURE.

C. J. Johnston and J. N. Finkelstein. *Pediatrics and Environmental Medicine, University of Rochester, Rochester, NY.*

Exposure to environmental pollutants may severely affect lung growth and development. Age appears to be a critical variable in the ability of the lung to cope with external stress. The timing and interaction between developmental events plays an important role in determining susceptibility to environmental perturbation. Sequential exposures to inhaled environmental pollutants may result in injuries/responses not predicted by evaluating exposures to individual pollutants. The following objectives tested the hypothesis in newborn, juvenile and adult mice during exposure to low levels of inhaled LPS and ozone: (1) Compare newborn vs adult responses to environmental agents to determine critical periods of development, (2) Examine how induction of lung epithelial damage or inflammatory cell activation followed by a second stimulus compares to responses after individual exposures in the postnatal lung. (3) Evaluate the role of early signaling cascades induced during acute injury. (4) Long term outcomes after early life exposure. We exposed C57Bl/6 mice varying in age from 2 thru 56 days. Mice were exposed to 0.5, 1 or 2.5 ppm ozone for 4 hours or LPS via inhalation and examined 0.5, 1, 2 or 4 hours post exposure. Tissue injury was determined by histological examination and analysis of epithelial and inflammatory markers. Proinflammatory, chemokine, antioxidant and early response genes were analyzed for changes in message abundance by RNase Protection Assay and immunohistochemical analysis. Our results demonstrate that critical time points exist during lung development to inhaled environmental pollutants and that differences exist in the maturation of inflammatory and epithelial defense mechanisms. Sequential exposures induced multiple signaling pathways and other responses which were age dependent and not predicted by individual exposures during postnatal lung development. Finally, early life exposures effected later life responses to ozone and LPS Exposure. Funded By: EPA Star PM Center R-827354 and P30 ES-01247.

S 573 OZONE-INDUCED INJURY IN THE IMMATURE/DEVELOPING NASAL AIRWAYS OF INFANTS.

J. R. Harkema[1], S. A. Carey[1], J. G. Wagner[1] and E. M. Postlethwait[2]. [1]*Pathobiology, Michigan State University, East Lansing, MI and* [2]*Environmental Health Sciences, University of Alabama at Birmingham, Birmingham, AL.*

Our laboratory has previously reported that acute exposures to ambient concentrations of ozone (O3), the principal oxidant pollutant in photochemical smog, cause rhinitis and epithelial necrosis in the anterior nasal cavity of adult macaque monkeys. Chronic, daily O3 exposures result in nasal epithelial remodeling (hyperplasia and mucous cell metaplasia) with attenuation of O3-induced nasal cytotoxicity and inflammation (adaptation) in these adult monkeys. Currently we are investigating the epithelial and inflammatory responses in the immature/developing nasal airways of infant rhesus monkeys that are episodically exposed to O3. In this presentation, we will provide an overview of 1) comparative nasal airway anatomy, 2) differences in structure and function of nasal airways in infants and adults, 3) state-of-art methods to determine ozone dose/response relationships in the nose, and 4) age-related susceptibility to nasal injury caused by ozone exposure. In recent studies, both acute and episodic exposures of ozone in infant monkeys caused marked rhinitis with epithelial necrosis and exfoliation. Unlike the nasal mucosa in adults, the immature/developing nasal mucosa of infant monkeys did not adapt to

repeated ozone exposure resulting in sustained susceptibility to injury. This presentation will conclude with 1) a discussion of the possible reasons why infant monkeys are more susceptible than adults to the nasal toxicity of ozone, 2) what implications these results have on the risk of nasal toxicity in children exposed to ambient ozone, and 3) what future research is needed. Research supported by NIEHS PO1 ES011617

S 574 OZONE-INDUCED CHANGES IN CONDUCTING AIRWAYS IN NON-HUMAN PRIMATES.

M. V. Fanucchi. *School of Veterinary Medicine, University of California, Davis, CA.*

Chronic airway disease and decreased lung function in children exposed to ambient air pollution may be due to repeating cycles of injury and repair altering normal lung maturation. Although rodent models have yielded much mechanistic data regarding the effects of ozone on adult lungs, they are of limited use for studying alterations in conducting airway structure. Nonhuman primates are a more appropriate model because their airway structure, epithelial cell composition (including abundant mucous cells) and postnatal lung development is more similar to humans than are rodent models. The distal portion of the airway tree in nonhuman primates is similar to that described in humans consisting of a terminal bronchiole followed by several generations of respiratory bronchioles (small airways with alveolar outpocketing in the walls) opening into the alveolar region. This region has been shown to be a critical target zone for oxidant air pollutants. This study was designed to test whether repeating cycles of injury and repair caused by episodes of ozone exposure lead to chronic airway disease and decreased lung function by altering normal lung maturation. Compared to filtered-air exposed control infants, ozone exposed animals had four fewer non-alveolarized airway generations, hyperplastic bronchiolar epithelium, and altered smooth muscle bundle orientation in terminal and respiratory bronchioles. These results suggest that episodic exposure to environmental ozone compromises postnatal morphogenesis of tracheobronchial airways.

S 575 STRUCTURAL AND FUNCTIONAL CONSEQUENCES OF OZONE INHALATION ON ALVEOLAR MORPHOGENESIS.

D. M. Hyde, N. K. Tyler, L. F. Putney and M. V. Avdalovic. *California National Primate Research Center, University of California, Davis, CA.* Sponsor: L. Van Winkle.

Oxidant air pollutant exposure in early childhood, with ozone as the key oxidant, has been linked to significant decrements in pulmonary function in young adults and exacerbation of airway remodeling in asthma. Since postnatal alveolar morphogenesis shows a rapid growth phase during the first two years of life, we exposed infant rhesus monkeys to biweekly 5 day cycles of ozone with or without house dust mite allergen from 1 to 6 months of age followed by a 6 month filtered air recovery period. At one year of age ozone-exposed monkeys had a significant increase in lung compliance, more, but larger, alveoli and an increase in the volume of the right middle lung lobe compared with controls. Ozone and allergen-exposed monkeys had no change in lung compliance, but decreased alveolar number, increased alveolar volume and a decrease in the volume of the right middle lung lobe compared with controls. Loss of alveoli was accompanied by increases in eosinophils compared with controls. Long-term inhalation of ozone during the first 6 months postnatal life alters alveolar morphogenesis and pulmonary function in a manner that persists for 6 additional months. The relationships among interalveolar septal cell destruction and apoptosis, oxidant injury and inflammation are emerging as key processes in the alteration of alveolar morphogenesis resulting from exposure to ozone.

S 576 STEM CELLS: NEW TOOLS FOR NEUROTOXICOLOGISTS.

T. J. Shafer. *Neurotoxicology Division, NHEERL, ORD, U.S. EPA, Research Triangle Park, NC.*

In the last decade, significant progress has been achieved in developing models of neuronal stem and precursor cells. These efforts have been directed primarily towards treatment of neurodegenerative disorders, restorative therapy for spinal cord injuries and other clinically-oriented issues. Stem and neuroprogenitor cells provide a readily available supply of human neurons, glia and oligodendrocytes, and may represent more accurate models of neuronal function and developmental processes than tumor-derived cell lines. As such, they have many potential uses for toxicologists, including mechanistic studies, safety pharmacology, and predictive toxicity screening. To date, the use of neural stem and progenitor cells for toxicity studies has been limited, but will continue to grow as these model systems become more readily available. This symposium will focus on uses of stem and neuroprogenitor cells in toxicology, including as models for investigative or mechanistic toxicology studies of developmental toxicity and potential uses in high-throughput screening of drugs and chemicals for neurotoxicity. This is an abstract for a proposed presentation, and does not represent Agency policy.

S 577 STEM CELL ASSAY FOR DEVELOPMENTAL NEUROTOXICITY.

D. M. DeGroot[1], S. Schulpen[1], H. Wortelboer[1], A. Freidig[1], J. Lammers[1], A. Wolterbeek[1], R. Westerink[2], A. Seiler[3] and J. Burgsteden[1]. [1]*TNO Quality of Life, Zeist, Netherlands,* [2]*IRAS, Utrecht, Netherlands and* [3]*ZEBET, Berlin, Germany.* Sponsor: V. Feron.

An *in vitro* test with stem cells is studied as a model to test potential Developmental Neurotoxicity of (un)characterized compounds. Embryonic stem cells from the blastocyst of the 129S2/SvPas mouse are forced to differentiate into neurons, astrocytes and oligodendrocytes through processes like proliferation, migration and differentiation, thereby mimicking neural development *in vivo*. The central hypothesis is that neurotoxicity *in vitro* can be determined by assessment of the absolute numerical relationship between the resulting celltypes in a compound treated culture, as opposed to an untreated culture (dose-dependent). Variations in culturing the stem cells resulted in distinct cells and many processes at cell densities of 175-225 thousand. A global distinction between dead and alive cells is made with acridine orange staining. The cells are characterized by their morphological appearance: 1) unstained 2) generally stained (H&E), or 3) selectively stained (immunohistochemically): beta 3-tubuline (neurons), O4 (oligodendrocytes), GFAP (astrocytes). Treatment of the test system with the thyroid hormone T3 led to an increase in oligodendrocytes. A problem with cell ratios is that one never knows whether the nominator or the denominator in the ratio changed, unless absolute cell numbers are known. Stereological principles and a computer aided stereological toolbox system (also succesful in *in vivo* developmental neurotoxicity [1, 2]) are used to normalize cell numbers and to overcome this problem. A quantitative *in vitro* method like this early provides valuable information about specific site(s) of action of a compound on the developing brain. We aim to test a selection of chemicals and will look for possible relations between the *in vivo* effects and the effects found on the individual processes and/or shift in cell types *in vitro*. [1] De Groot et al. Environm. Toxicol. Pharmacol. (2005) 19:745-755 [2] De Groot et al. Reproductive Toxicology (2005) 20:417-432. Sponsored by the Dutch Ministery Health, Welfare and Sports

S 578 HUMAN NEUROSPHERES IDENTIFY THREADS FOR BRAIN DEVELOPMENT.

E. Fritsche, J. E. Cline, K. Gassmann, J. Heinrichs, M. Moors, T. D. Rockel, T. Schreiber and J. Abel. *Toxicology, Institut für umweltmedizinische Forschung, D√É¬ºsseldorf, Germany.* Sponsor: T. Shafer.

Current developmental neurotoxicity testing (DNT) in rodents *in vivo* possesses two major obstacles: the requirement of large amounts of animals and the uncertainty of extrapolating the data to humans. Therefore, we have established a human *in vitro* model for DNT based on normal human neural progenitor (NHNP) cells which are cultured as proliferating neurospheres. On coated surfaces, NHNP cells migrate radially out of the sphere and thereby differentiate into the major neural cell types of the brain.

Proper brain development is assured by the interplay of cell proliferation, apoptosis, differentiation and migration. Therefore, we developed *in vitro* methods, which allow detecting effects of chemicals on these fundamental processes.

E.g., Immunocytochemical analyses of differentiated NHNP cells reveal that cAMP induces neuronal differentiation, while methylmercury causes an increased formation of astrocytes. Oligodendrocyte development was promoted by thyroid hormone and inhibited by exposure to lead indicating that we are able to influence cell determination of NHNP cells.

Measurements of the track that these cells wander during their differentiation revealed that exposure to mercury compounds, ethanol or carbaryl led to an inhibition of NHNP cell migration. Investigations of the mechanisms underlying NHNP cell migration show regulation via Mitogen Activated Protein Kinase ERK1/2-dependent and ERK1/2-independent pathways, which are regulated by PKC, EGFR and src family kinases. Therefore, interference of chemicals with these target molecules may cause disturbed cell migration and impair brain development in humans. Here, we report that primary neural cells of human origin can be utilized to evaluate developmental neurotoxicity potential of given chemicals *in vitro*, thereby reducing animal consumption and costs. Furthermore, these cells provide a unique view into the molecular mechanisms of toxicity in human brain. Therefore, we suggest that NHNP cells are an apt human *in vitro* model for DNT testing.

S 579 EFFECTS OF NEUROTOXICANTS ON PROLIFERATION AND VIABILITY OF IMMORTALIZED HUMAN CORTICAL NEURAL PROGENITOR CELLS.

J. M. Breier[1, 2], W. R. Mundy[2] and T. J. Shafer[2]. [1]*Toxicology, University of North Carolina, Chapel Hill, NC and* [2]*Neurotoxicology Division, U.S. EPA, Research Triangle Park, NC.*

Use of human neural progenitor cells (hNPCs) for predictive neurotoxicity screens has not been widely examined. As an initial effort toward evaluating hNPCs as a model for screening for developmental neurotoxicants, we characterized undifferentiated ReN CX cells and tested neurotoxicants for effects on cell proliferation and viability 4 hours post treatment. ReN CX cells are an immortalized NPC line generated from a 14-week sample of human cortex that differentiates into neuronal and glial populations. The cells expressed nestin and Sox-2, markers of multipotency, in the presence of EGF and FGF-2. Cell proliferation and viability were assessed via bromodeoxyuridine (BrdU) incorporation and propidium iodide staining using an automated, high content approach (Cellomics Arrayscan). Removal of EGF and FGF-2 from the expansion media, which initiates differentiation, decreased BrdU incorporation 12% (4 hours) and 41% (24 hours) compared to controls without affecting viability. The anti-proliferatives aphidicolin and hydroxyurea were tested as positive controls. Aphidicolin (IC50=0.21 μM) more potently inhibited proliferation in ReN CX cells compared to hydroxyurea (IC50=23.81 μM), and neither affected viability up to 30 μM. The known neurotoxicants methylmercury and trans-retinoic acid (1 nM – 100 μM) inhibited proliferation in a concentration-dependent manner, however, only methylmercury inhibited proliferation at concentrations (1-3 μM) that did not affect viability. Methylmercury selectively inhibited cell proliferation in this model. By contrast, three non-neurotoxic compounds (acetaminophen, amoxicillin, diphenhydramine; 1 nM – 100 μM) were without effect on proliferation or viability up to 30 μM. These data demonstrate that ReN CX cells have characteristics of neural progenitor cells and that proliferation can be assessed using high-throughput methods. Ongoing studies are testing additional sets of known neurotoxic and non-neurotoxic compounds to determine the utility of this approach as a screen. This is an abstract for a proposed presentation, and does not represent Agency policy.

S 580 THE NRF2-ARE PATHWAY: IDENTIFICATION OF NEUROTOXIC AND/OR NEUROPROTECTIVE CHEMICALS.

J. Johnson[1, 2, 3], D. Johnson[1, 2], J. Li[1], M. Calkins[2], M. Vargas[1] and M. Emborg[4]. [1]*Pharmaceutical Sciences, University of Wisconsin, Madison, WI,* [2]*Molecular and Environmental Toxicology Center, University of Wisconsin, Madison, WI,* [3]*Neuroscience Training Program, University of Wisconsin, Madison, WI and* [4]*Wisconsin National Primate Research Center, University of Wisconsin, Madison, WI.*

Oxidative stress is thought to contribute to a variety of neurodegenerative diseases and oxidative stress pathways are activated by a wide variety of neurotoxic compounds. The Nrf2-ARE pathway is responsible for activation of a number of antioxidant defense genes. We have demonstrated neuroprotective efficacy of neural progenitor cells overexpressing Nrf2 and have developed several transgenic models of Nrf2 overexpression as well as ARE-reporter mice that are sensors of oxidative stress. For example, transplantation of Nrf2 overexpressing neuroprogenitor cells protected striatial neurons from malonate-induced neurotoxicity. This presentation will discuss these transgenic mice and their use in a variety of neurotoxic models of neurodegeneration. In addition, primary neuronal cultures and neural progenitor cells derived from the ARE-reporter mice have been used to screen for chemical activators of the Nrf2-ARE pathway. Pioglitazone, an FDA approved drug, was identified as an activator of Nrf2 and neuroprotection was completely dependent upon Nrf2 since no protective effect was seen in Nrf2-/- primary cortical neuronal cultures. Blood-brain-barrier penetration and activation of the Nrf2-ARE pathway in brain was demonstrated using the ARE-hPAP reporter mouse. Subsequent studies in non-human primate models of Parkinson's disease demonstrated that MPTP toxicity was reduced when pioglitazone treatment was initiated 24 hours after administration of MPTP. These data strongly support the concept that chemicals shown to activate the Nrf2-ARE pathway in vitro, followed by in vivo validation experiments has the potential to identify novel therapeutic approaches for Parkinson's disease as well as other neurodegenerative diseases. *NIEHS ES10042, ES08089, State of Wisconsin, ALSA, Packard Center for ALS Research, HDF, MJFox Foundation.*

W 581 GETTING THE MOST OUT OF MODEL ORGANISM DATABASES: FROM THE BASIC TO THE COMPLEX.

S. M. Bello. *Mouse Genome Informatics, The Jackson Laboratory, Bar Harbor, ME.*

Model organisms are surrogates for studying normal and disease-related biology as well as extrapolating to potential human responses to toxicants. The selection of a model depends on the species' or strains' characteristics and relies on in-depth knowledge of the model's biology. Today, the amount of data available for any one model organism greatly exceeds the amount of time researchers have to spend tracking down these data. This is especially true for those organisms (zebrafish, rat and mouse) with significant biological knowledge bases that are the subject of whole genome sequencing and large-scale projects in mutagenesis, gene expression and biological networks. To deal with the data overload, model organism databases have been developed to capture and integrate these data, provide quick access to fundamental data on genes and strains and enable complex biological questions to be addressed. The integration of phenotypic, functional, and genetic data combined with data mining and visualization tools allows users to quickly extract information that would otherwise have taken days or weeks to find. However, the differences between databases complicates the process of extracting this information. The types of data as well as the balance and emphasis of similar data types differs between databases, reflecting the different research strengths of each model organism. In addition, each database has its own unique set of data mining and visualization tools designed to help analyze and understand the data from that species. Thus, determining how to use a specific database to address your question, whether it is to find out what is known about a gene in that species, identify the best strain or mutant line for an experiment or generate a list of potential candidate genes, may be a daunting task. A researcher armed with knowledge about their model organism database's strengths and weaknesses is better able to extract the answers needed to push the research forward.
Overviews of the represented databases will be given and internet access will allow participants to work through example searches or try queries relevant to their own research with experts on hand to answer questions.

W 582 ZFIN, THE ZEBRAFISH MODEL ORGANISM DATABASE.

M. Westerfield and . and the ZFIN Staff. *ZFIN, University of Oregon, Eugene, OR.* Sponsor: S. Bello.

The Zebrafish Information Network (ZFIN, http://zfin.org) is a web-based community resource that supports research with zebrafish. ZFIN provides a centralized, integrated repository and query interface for zebrafish research data, including mutants, genes, genetic markers, mapping panels, links to other genomic resources, publications and community resources such as meeting announcements and contact information. Researchers use ZFIN to obtain information about genes and genetic pathways involved in development, organ function, behavior and disease. ZFIN provides extensive information about gene function, including expression, mutant phenotypes and models of human diseases.
ZFIN provides extensive online documentation for using ZFIN and individualized user help via email or telephone. ZFIN also provides customized searches and data reports.
ZFIN is funded by the National Human Genome Research Institute of the National Institutes of Health.

W 583 THE RAT GENOME DATABASE: INTEGRATED DATA PLATFORM FOR RESEARCH.

M. Shimoyama, V. Petri, S. Laulederkind, J. Smith, R. Nigam, G. Kowalski, D. Li, J. DuPons, S. Twigger, A. Kwitek and H. J. Jacob. *Human and Molecular Genetics Center, Medical College of Wisconsin, Milwaukee, WI.* Sponsor: S. Bello.

The Rat Genome Database (RGD, http://rgd.mcw.edu/) is the premier international data repository and integration platform for rat genetics and genomics, biological findings from rat research and strain information. RGD also provides comprehensive ortholog reports for mouse and human genes as well as QTLs for these species. In addition, information on genomic and other map positions, nucleotide and protein sequences, protein domains and structures, and links to all major sequence, protein and pathway databases with appropriate identifiers make RGD a one-stop resource for all rat researchers.
Controlled vocabularies are used to facilitate data mining and integration across disparate data types. The Gene Ontology (GO) is used to represent molecular function, biological process and cellular component information. Controlled phenotype, pathway and disease data are also used to annotate genes, QTLs and strains. Text notes and descriptions supplement and amplify this information.
The tools RGD provide the user with multiple ways to access, integrate and analyze data. General and advanced searches allow the user to retrieve data via disease terms, phenotypes, function, chromosome and map positions, identifiers, and authors' names. Specialized portals for cardiovascular, neurological diseases give the user easy access to multiple data types – diseases, genes, orthologs, phenotypes, pathways and strains. Bulk downloads from various types of search results provide an easy way for users to save information on multiple data types. Data visualization

via GBrowse and GViewer provide a view of biological information within the genomic context while VCMap provides a comparative view of genes and QTLs across rat, human and mouse.
RGD not only provides the most comprehensive resource for users but also provides documentation and customized datasets and user help to ensure researchers obtain both the data and analyses necessary to advance their studies.
RGD is supported by NIH/NHLBI grant HL64541.

W 584 THE MOUSE GENOME DATABASE: A SHORT USER'S GUIDE.

S. M. Bello, C. L. Smith, H. Dene, D. L. Burkart, L. L. Washburn, I. Lu, M. Tomczuk, A. Anagnostopoulos, B. Richards-Smith, M. Updegraff, H. Onda, R. Babiuk, M. Knowlton and J. T. Eppig. *Mouse Genome Informatics, The Jackson Laboratory, Bar Harbor, ME.*

The Mouse Genome Informatics Database (MGI, http://www.informatics.jax.org) is the free international resource for the genetics, genomics and biology of the laboratory mouse. MGI includes extensive descriptions of mouse mutant phenotypes, relationships of genes and gene products to molecular function, biological process, and cellular component using controlled vocabularies (the Mammalian Phenotype (MP) Ontology and Gene Ontology (GO)). These annotations are integrated with other biological data, including mouse nucleotide and protein sequences, SNPs, developmental gene expression, chromosomal and genomic location of genes, and mammalian gene orthologs. MGI allows users to search, compare, and analyze data in the context of multiple data types.
MGI provides a variety of ways to access data including; a quick search box for simple searches, query forms for posing more complex questions, vocabulary browsers to find phenotypic or gene function related information, a batch query form to retrieve data for large lists of genes, and data visualization tools such as GBrowse and comparative maps to aid in data analysis. With this array of tools users can: find extensive information about their gene of interest; pose complex questions such as "What genes are expressed in the cardiovascular system, have mutations that result in abnormal vascular development, and are known to be involved in mesoderm development?"; visualize all system level phenotype annotations within a genome region; or identify all strains with known SNPs within a specified genome region. Integration and the use of structured vocabularies for annotations assists in robust and accurate data mining when posing either simple or complex questions using any of the tools available at MGI.
MGI provides user documentation, individualized user help, and customized user data searches (contact via email at mgi-help@informatics.jax.org)
The Mouse Genome Database (MGD) at MGI is supported by NIH/NHGRI grant HG000330.

W 585 MOUSE PHENOME DATABASE (MPD): A RESOURCE AND RESEARCH TOOL.

M. A. Bogue, T. P. Maddatu, C. J. Bult and S. C. Grubb. *Mouse Phenome Project, The Jackson Laboratory, Bar Harbor, ME.* Sponsor: S. Bello.

The Mouse Phenome Database (MPD; http://www.jax.org/phenome) is a repository of quantitative phenotypic data on commonly used, genetically diverse inbred strains of mice and their derivatives. Mice are tested by experts in the community using a strain survey approach under controlled, well-defined conditions. Strain characteristics data are contributed to MPD for annotation, storage, and worldwide access. MPD also contains a large collection of genotypic data (primarily SNPs) consolidated from community sources.
MPD offers a powerful platform for data analysis and in silico hypothesis testing. Electronic access to centralized strain data enables researchers to choose appropriate strains for many systems-based research applications, including physiological studies, drug and toxicology testing, mapping genes, and modeling disease processes.
MPD provides several ways to find phenotypic data of interest. In addition to routine keyword searches users may find data by phenotype (browse by category), test apparatus, protocol, strain or strain set, contributing investigator, or intervention (such as hi-fat diet or cadmium exposure). Raw data sets are available for bulk downloading, and tabular summaries (statistical analysis) can be viewed on screen or downloaded as an Excel file. Various plotting options are available, and measurements of interest can be analyzed though the MPD tool wizard. Correlation matrices, scatterplots, outlier strain identification, strain comparisons, sex comparisons, and other tools are available. MPD offers a SNP wizard for accessing mouse SNPs for ~ 10 million genome-wide locations. SNP records are linked to MGI.
MPD provides user support at phenome@jax.org.
The Mouse Phenome Database (MPD) at The Jackson Laboratory is supported by the National Heart, Lung, and Blood Institute, National Human Genome Research Institute, and National Institute of Mental Health.

W 586 MIXTURE EXPOSURE TO METALS/METALLOIDS AND RELATED HEALTH EFFECTS.

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Exposure of human populations to mixtures of metals (lead, cadmium, mercury) and metalloids (arsenic) are common under both occupational and environmental situations such as Superfund sites. The routes of exposures to these mixtures may include inhalation, ingestion or absorption across the skin and result in a number of deleterious health effects. Particle exposures of rodents to binary semiconductor compounds such as gallium arsenide and indium arsenide have demonstrated a number of compound – specific changes in proteomic and metabolomic biomarkers. Studies of human populations in China with combined exposures to cadmium and arsenic have demonstrated marked increases in renal toxicity with regard to proteinuria biomarkers. Factorial design studies in rats exposed to low dose mixtures lead, cadmium and arsenic have shown increased oxidative stress in kidneys and changes in a number of other target organ system biomarkers as a function of duration of exposure. Interaction studies between lead and dietary calcium have demonstrated a strong influence of calcium intake on the gastrointestinal absorption of lead and hence risk of toxicity. Selenium exposure has been shown to influence the toxicity of inorganic mercury but not methyl mercury. Data from these studies provide clear evidence for the need to understand interactions among components of metallic mixture exposures in order to improve human health risk assessments.

W 587 INTERACTIVE EFFECTS ON MOLECULAR BIOMARKERS FOLLOWING EXPOSURE TO BINARY III-V SEMICONDUCTOR COMPOUNDS: AN OVERVIEW OF *IN VIVO* AND *IN VITRO* STUDIES.

B. A. Fowler[1], E. A. Conner[2] and H. Yamauchi[3]. [1]*Division of Toxicology and Environmental Medicine, ATSDR, Atlanta, GA,* [2]*Laboratory of Experimental Caricnognesis, NCI, Bethesda, MD and* [3]*Pharmaceutical Sciences, Kitasato University, Kitasato, Japan.*

The semiconductor industry is global in nature and employs a large number of female as well as male employees. These demographic data require consideration of gender - specific differences in sensitivity to chemical agents widely used in this industry. In vivo exposures to 3-4 uM particles of the binary semiconductors GaAs and InAs in rodents demonstrated compound – specific target organ system effects as monitored by molecular proteomic and metabolomic biomarkers. These biomarker responses were also found to show specific time-dependent characteristics following exposure. Complementary in vitro studies using hamster and human primary kidney cell cultures demonstrated similar proteomic responses between the 2 species but that these responses were found to vary as a function of gender. These data indicate that risk assessments for humans exposed to these binary semiconductor compounds may take advantage of the similarities in biomarker responses in both rodent and human target cell populations and should additionally take gender-specific differences in into account.

W 588 MIXED EXPOSURES TO CADMIUM AND ARSENIC AND RELATED DOSE-RESPONSE RELATIONSHIPS IN HUMANS.

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Adverse renal effects of cadmium are well documented in humans and animals, but only limited data are available on such effects from exposure to inorganic arsenic. The present studies were performed on human population groups in two areas in China where there were mixed exposures to inorganic arsenic and cadmium from contamination of air, food and drinking water in the general environment. Comparisons were made with population groups in nearby non-contaminated areas.
Results: In both exposed areas there were statistically significant increases compared to the control areas of Urinary Total Arsenic (UTAs), Urinary Cadmium (UCd), and biomarkers of renal dysfunction, i. e. urinary levels of beta.2 microglobulin, N-acetyl- beta-D-glucosaminidase, retinol binding protein and albumin. In the area where there was most pronounced exposure to inorganic arsenic (and some exposure to Cd) there was a clear dose-response pattern both in relation to UTAs and UCd and the biomarkers of renal dysfunction. There was a clear interaction effect of the combined exposure (as indicated by UTAs and UCd) enhancing the effects on the kidney. In the other area where cadmium exposure was predominant, renal ef-

fects were related to UCd,but not to UTAs. However there was a statistically significant relationship between urinary inorganic arsenic and urinary beta-2-microglobulinuria among women and an interaction between Cd and IAs was indicated. Conclusion: These studies demonstrated clear and strong interaction between Cd and As in inducing renal dysfunction among humans in one area in China and indicated a similar interaction in another area. It is urgent to perform further studies in these and other areas in order to confirm these interactions and also to study potential interactions among these exposures regarding other adverse health effects.

W 589 EXPOSURE TO PB, CD, AND AS MIXTURES POTENTIATES THE PRODUCTION OF OXIDATIVE STRESS PRECURSORS: 30-DAY, 90-DAY, AND 180-DAY DRINKING WATER STUDIES IN RATS.

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Exposure to chemical mixtures is a common and important determinant of toxicity. To date, few in vivo mixture studies have been conducted to understand the impact of chemical mixtures compared to single chemicals at low dose levels. Interactive effects of lead, cadmium, and arsenic were evaluated in 30-, 90-, and 180-day drinking water studies in rats designed to test the hypothesis that ingestion of lead, cadmium, and/or arsenic mixtures at Lowest Observed Effect Levels (LOELs) results in increased levels of the pro-oxidant aminolevulinic acid (ALA), iron, and copper. LOEL levels of Pb, Cd, and As mixtures increased levels of multiple oxidative stress precursors such as ALA, copper, and iron, as evidenced by statistically significant increases in urinary ALA in the 30-day study (all groups but PbxCdxAs). ALA increases among mixture groups from the 30-day study were followed by statistically significant increases in kidney copper in subsequent 90- and 180-day studies. Statistical evidence of trace element interaction was identified for six biologically relevant variables: blood aminolevulinic acid dehydratase (ALAD), kidney ALAD, urinary ALA, urinary iron, kidney iron, and kidney copper. The current investigations underscore the importance of considering interactive effects of common toxic agents such as Pb, Cd, and As. Interactions between known toxic trace elements at biologically relevant concentrations shown here demonstrate a clear need to rigorously review health risk assessment methods since such exposures may commonly occur within complex mixtures.

W 590 MULTIPLE CONTAMINANT METALS AND NUTRITION.

K. R. Mahaffey. *Exposure Assessment and Coordination and Policy, U.S. EPA, Washington, DC.*

Both the nature of the toxic effect and the shape of the dose-response curve characterizing metal toxicity can be affected by nutritional status. These concepts will be illustrated by examples from the inorganic metals lead and mercury. Calcium status is known to alter fractional intestinal absorption, tissue distribution, and toxic effects of lead in multiple animal species and people. Iron status substantially modifies the dose of lead producing hematological effects. Inorganic mercury tissue distribution with associated hepatic and renal toxicities are shown to be modified by selenium status. By contrast the organo-mercurials (demonstrated by studies with methylmercury, the most ubiquitous member of this series) present a distinctly different situation. Despite many attempts to alter methylmercury toxicity, especially to the nervous system, through nutritional manipulation the demonstrated efficacy of such interventions to the intact animal remains marginal. Human exposure ranges for both the toxicants, as well as the nutrients, and the likelihood of nutritional factors being influential through sustainable human diets will be addressed.

W 591 WHERE THE RUBBER MEETS THE ROAD: CURRENT APPLICATION OF GENOMIC TOOLS IN PRODUCT DEVELOPMENT AND DECISION MAKING IN THE CONSUMER PRODUCT, PHARMACEUTICAL, AND CHEMICAL INDUSTRIES.

R. S. Thomas[1] and G. P. Daston[2]. [1]*The Hamner Institutes for Health Sciences, Research Triangle Park, NC and* [2]*Central Product Safety Evaluation, Procter & Gamble, Cincinnati, OH.*

The use of genomic tools to broadly survey the transcriptional response of an organism or cell began over a decade ago. The toxicology community initially embraced the technology with the hope that it would allow rapid hazard identification and provide mechanistic insights into how environmental chemicals and pharmaceuticals exert their adverse effects. However, the direct application of genomic technology has proven to be challenging for the both toxicology and regulatory communities. Issues such as reproducibility, the ability to link transcriptional changes to phenotypic effects, and the conservative nature of safety assessment have all hindered the acceptance and wide-spread application of genomic tools. With the maturation of the technology, the toxicology and regulatory communities now have a better grasp on how these tools can be applied in product development and decision making in the context of safety assessment. The purpose of this symposium is to offer practical examples of how genomic technologies are being applied within a broad range of industries together with perspectives from the EPA and FDA on how these data are currently being used in regulatory decision-making.

W 592 GENOMIC TECHNOLOGY AND ANALYSIS FOR ASSESSING TOXICITY AND RISK IN THE CONSUMER PRODUCTS INDUSTRY.

G. Daston and J. Naciff. *Miami Valley Labs, Procter & Gamble, Cincinnati, OH.*

Genomic technologies have the potential to make a significant impact the way in which hazard and risk assessment are done. Research over the past several years in many labs, including ours, has demonstrated that different modes of toxic action elicit specific gene expression profiles in responsive tissues. These profiles can be used to predict toxicity potential, and can be used as the basis for tailoring a toxicity testing strategy based on mode of action, rather than applying the one-size-fits-all approach now used. Microarray analysis may also be useful as the basis for in vitro screens for toxicity, as the readout (changes in gene expression) is the same in in vitro systems as in the complex organisms now used to evaluate toxicity. Genomic technologies also can be used as the basis for biologically-based dose-response models, which have the potential to improve low-dose extrapolation and make risk estimation at ambient exposure levels less uncertain. Finally, microarray analysis combined with informatics can provide valuable information about the role that specific genes or families of genes play in biological processes that are critical for toxic response. Each of these applications can be illustrated using examples from our lab using steroid hormone ligands as toxicologic probes. We have demonstrated that gene expression response is mode-of-action-specific, but is not necessarily tissue-specific or life-stage specific. We have demonstrated the behavior of estrogen-responsive genes across six orders of magnitude of estrogen dosage, illustrating the robustness of gene expression for dose-response applications. Finally, we have analyzed the temporal expression of genes to elucidate the molecular underpinnings of the uterine response to an estrogenic stimulus. Although these technologies are not yet ready for routine use in toxicology testing, they are already a useful adjunct to safety studies and will continue to play a significant role in improving hazard and risk assessment.

W 593 APPLICATION OF GENOMICS TESTING TO NEW PRODUCT DEVELOPMENT.

G. M. Rusch. *Toxicology & Risk Assessment, Honeywell International, Morristown, NJ.*

Due to regulatory pressure, Honeywell had a need to complete a full evaluation of a potential new fluid for mobile air conditioning systems within a 3.5 year time frame. Typically this program would require 5 to 7 years. Using a genomics approach instead of the traditional "2-year inhalation carcinogenicity study" we were able to complete the full program in less than 3 years. Not only did the assay appear, based on control chemicals, to be as least as predictive as the "bioassay", it also required less than 5% of the material that the bioassay would have needed thus allowing a much quicker initiation. Validation of the study was accomplished by the incorporation of structurally similar positive and negative controls. These results are being submitted to regulatory agencies in both the U.S. and E.U.

W 594 THE USE OF GENOMIC INFORMATION, KNOWLEDGE TOOLS AND INTELLIGENCE NETWORKS FOR PRECLINICAL DECISION MAKING IN THE PHARMACEUTICAL INDUSTRY.

J. A. Reynolds[1, 3, 2]. [1]*JAReynolds & Associates, Madison, CT,* [2]*Safety Sciences & Translational Research, The Hamner Institutes for Health Sciences, Research Triangle Park, NC and* [3]*BioWisdom Ltd., Cambridge, United Kingdom.*

The transformation of biological methods currently utilized by safety scientists has been propelled by an array of rapidly emerging enabling technologies. This evolution has resulted in our ability to readily generate millions of data points around biological processes. The ability to integrate, manage and query this plethora of data

is essential to knowledge creation and the underpinning of more effective safety-related decision making. The ideal objective of data management is to include ALL available information in order to provide a comprehensive and unbiased knowledge substrate across which analysis and research can be performed most efficiently. Intelligence networks derived from various ontologies present integrated information through a variety of analytical and visualization tools, ranging from pathways, chemoinformatic, toxicoinformatic, statistical, and document analysis systems through to more specialized competitive intelligence and in-licensing workflows. In addition, there is currently a broad range of commercially available software applications that can provide an enterprise data integration platform across hundreds of disparate databases. These databases can be viewed through a single portal or alternatively, by more sophisticated desk-top applications that enable either large data sets or information contained in published literature to be visualized, clustered and annotated. These platforms and technologies can be applied to assist safety scientists in understanding the basic biology of adverse events or toxicity mode of action that enables improved and more effective decision making and risk assessment.

W 595 AN ARRAY OF TOXICOGENOMICS EFFORTS AT FDA.

W. Tong. *NCTR, Jefferson, AR.* Sponsor: R. Thomas.

Toxicogenomics (TGx) is identified in the FDA Critical Path document as a major opportunity for advancing medical product development and personalized medicine. An array of FDA efforts on TGx has been taking place at the inter-center and cross-community collaborative levels. Specifically, FDA issued guidance to industry on pharmacogenomics data submission. The guidance defines a novel mechanism entitled Voluntary Genomics Data Submission (VGDS) whereby the sponsor is able to interact with the agency in the early stage of drug development by submitting pre-clinical data on a voluntary basis. To facilitate this process, an integrated FDA bioinformatics tool, called ArrayTrack, developed at NCTR/FDA, is being refined as a review tool for managing, analyzing and interpreting this exploratory data i.e. genomic, proteomic and metabolomic data, from both clinical and non-clinical data submissions. To further understand the TGx technology in the regulatory context, a MicroArray Quality Control (MAQC) project has been initiated. This is a community supported project led by the FDA to address various issues associated with DNA microarrays, a critical technology used in the generation of TGx data. The lessens learned from both VGDS and MAQC are paving the way for development of a Best Practice Guidance Document for future voluntary as well as regular submissions of TGx data to the FDA. ArrayTrack is an integral part of VGDS and MAQC. Together these capabilities provide the Agency with an integrated bioinformatics infrastructure to support data management, analysis and interpretation. ArrayTrack also serves as a vehicle to translate the Best Practice Guidance Document into routine application for regulatory review and decision making.

W 596 USE OF GENOMICS DATA AT THE U.S. EPA FOR PREDICTIVE AND MECHANISTIC TOXICOLOGY.

D. J. Dix. *National Center for Computational Toxicology, U.S. Environmental Protection Agency, Research Triangle Park, NC.*

From both in vivo and in vitro model systems, the EPA is examining genomics data and incorporating it into predictive and mechanistic models of toxicity. One example of this is EPA's ToxCast research program, which is developing the ability to forecast toxicity based on bioactivity profiles that incorporate genomics data. Examples from in vivo toxicogenomic studies of pesticides and industrial chemicals, wherein the data has contributed to a fuller understanding of mode of action, will also be presented.

IS 597 FISH: BRAIN FOOD OR BRAIN POISON? A SPECIAL REGIONAL INTEREST SESSION.

E. Faustman[1] and R. Lorenzana[2]. [1]*Institute for Risk Analysis and Risk Communication, Seattle, WA and* [2]*U.S. EPA, Seattle, WA.*

The Puget Sound Georgia Basin ecosystem, extending from the Campbell River in Northern British Columbia, Canada to the Nisqually River in Central Western Wa in the US is one of the most ecologically diverse in North America. The health of this ecosystem is a cornerstone of the region's quality of life and vibrant economy. Current population in this region is over 6 million people with projections that by 2020 to over 10 million. The area is ethnically diverse with increasing representation of Asian, Latin and European cultures. The area is also home to many First Nation communities (Canada) and Tribal Nations (US side) with long established cultural, social, religious and resource-based traditions and customs within these local areas. However, this uniquely valuable region faces increasing pressures and the vital relationship between ecosystem health, human health and ocean are at risk. Of particular challenge to the scientific community is the complexity and interrelatedness of these pressures for predicting and protecting human and ecosystem health. This symposium will explore these relationships. Case examples from the region will describe ecosystem challenges in relationship to persistent organic pollutants and marine mammal health and characterize risks for high fish and shellfish consuming human populations. The regional public health risk assessment dilemmas for encouraging consumption of "heart and brain healthy" seafoods will be discussed in relationship with risk messages warning of eating contaminated seafoods that may contain neurodevelopmentally toxic or cancer causing substances. The importance of understanding the dynamics of naturally produced toxins such as those from harmful algal blooms, the significance of microbial contaminants (such as vibrio) and presence of persistent environmental pollutants that bioaccumulate will illustrate the need for integrated public health relevant risk assessments and prevention measures for this region.

IS 598 STATE OF THE PUGET SOUND.

W. Ruckelshaus. *Puget Sound Nearshore Ecosystem Restoration Project, Salmon Recovery Funding Board, Seattle, WA.* Sponsor: E. Faustman.

The Puget Sound Partnership was designed to form an integrated new group that would address the serious challenges for our regional Puget Sound Ecosystem. These challenges include human population expansion to 10 million by 2010 and increasing demands for clean water, access to pristine shoreline and recreational activities and booming economy. How can we maintain our glorious cultural and ecosystem diversity? This talk with identify these challenges and the recent actions to address these concerns from a risk management context.

IS 599 OCEANS AND HUMAN HEALTH: LESSONS LEARNED FROM THE PUGET SOUND.

E. Faustman. *Institute for Risk Analysis and Risk Communication, Seattle, WA.*

The Ocean and Human Health Initiative from NOAA, NIEHS and NSF has spearheaded efforts to meet recent challenges to integrate public health and oceanography by evaluating common methods, context and constructs. Of particular interest for our region and for the University of Washington Ocean and Human Health Center has been developing an understanding of underlying mechanisms that define and shape these interactions. Three key areas of research focus has arisen from these considerations. First, a common parallel between oceanography and toxicology has been identified as both research disciplines are investigating gene environmental interactions. Foe example, questions concerning what triggers algal blooms to produce toxic compounds such as Domoic acid involves understanding both the environmental conditions and genetic variation of algal species in bloom events. Likewise, for public health, understanding what environmental conditions lead to contaminated shellfish and what human populations are sensitive are parallel gene environment based mechanistic considerations. Second, geographic context for these problems is needed however it is surprising that so little integrated or consistent data exists for shore, near shore and coastal areas. The Puget Sound has over 2,500 miles of coast with approximately 3 million people versus our ocean coastal regions of 500 miles and sparse populations. This has significant implications for optimizing biomonitoring programs. Third, a lack of common framework for answering questions on oceans and human health impedes our basic understanding and clarification of key mechanisms. This talk will explain how the Oceans and Human Health Initiative is addressing these challenges in or Pacific Northwest Region.

IS 600 MARINE ECOSYSTEMS AND HUMAN HEALTH IN PUGET SOUND.

U. Varanasi, J. Stein, T. Collier, W. Dickhoff and T. Hom. *NW Fisheries Science Center, NOAA Fisheries, Seattle, WA.* Sponsor: E. Faustman.

Just as human activity affects the health of the marine environment, so too does the state of the marine environment affect the health of humans living near marine systems. This is very evident in Puget Sound, where the human population is projected to double in the next few decades. The growing population will lead to increased chemical contaminant loadings to the waters and sediments of the Puget Sound basin unless we drastically modify our strategies for dealing with both point sources and non-point sources of pollution. The widespread increase in pollution will have consequences for human health, especially for children and seafood-dependent populations. In addition, increasing nutrient loadings and changing climate are already being linked to increasing incidences of harmful algal blooms in Puget Sound, including the occurrence of toxic species that were until now only seen in open coastal and oceanic waters. Moreover, population growth may lead to

increased loads of disease-causing bacteria, viruses, and protozoa (e.g. E. coli, norovirus, and giardia, respectively) from animal waste and malfunctioning sewage systems. These introductions, combined with increased nutrients and warmer temperatures, may create conditions that favor rapid bacterial growth in estuarine waters, including shellfish growing areas. These increased risks have already affected human health and well-being in the Puget Sound Basin (increased outbreaks of pathogenic bacteria in shellfish), but to date most of our management strategies have involved restrictions on human exposure to the affected portions of the ecosystem (e.g. fish consumption advisories, shellfish beach closures), and those strategies reduce public use and enjoyment of the Puget Sound which can have other consequences. Our research is aimed at improved forecasting, mitigation, and early warning to reduce these potential risks, with climate change as a backdrop. We must better understand the interplay between human health, societal values, and the health of Puget Sound, in order to protect and restore this unique ecosystem.

IS 601 MERCURY EXPOSURE IN A GROUP OF PUGET SOUND AREA ASIAN-AMERICAN WOMEN OF CHILD-BEARING AGE.

K. Marien. *Department of Health, Office of Environmental Health Assessments, Olympia, WA.* Sponsor: E. Faustman.

While there are many forms of mercury, methylmercury exposure through the consumption of seafood has received the most attention due to the association of exposure to methylmercury with adverse effects on the developing nervous system. Accordingly, the population of concern is represented by women of childbearing age, infants and children. In the US, the most prominent sources of exposure are diets consisting of fish and shellfish that contain methylmercury. Puget Sound area studies have shown that Asian Americans are among those who consume large amounts of fish and shellfish. Subjects who identified themselves as Japanese or of Japanese descent, were of childbearing age women and who were residing in the Puget Sound area were recruited through a local clinic in Seattle area from August to December 2005. Each subject was asked about their fish consumption behavior patterns using a fish consumption survey and was asked to provide various biometric samples at the time of the interview. The survey was designed to provide culturally appropriate descriptions of the seafood in Japanese to capture long term consumption patterns. For this study, data from the survey instrument along with mercury analysis data of locally purchased fish as well as blood mercury levels are being used to assess the extent of exposure within the community. Sixty-five blood samples were analyzed for total mercury. The median total mercury in blood was 1.9 ug/L (ppb) and the range was 0.12 -44.2 ug/L. The result of the fish consumption survey, the relationship between the survey and the mercury levels in blood indicate the importance of population specific data in assessing health risks.

IS 602 DEFINING CULTURAL WELL-BEING FOR TRIBAL NATIONS: GOING BEYOND ACCEPTABLE LEVELS OF RISK.

J. Donatuto. *Office of Planning and Community Development, Swinomish Indian Tribal Community, LaConner, WA.*

This presentation will be to provide insight on the Swinomish Indian Tribal Coummunity's new approaches for evaluating bioaccumulative toxics in shellfish. These new approaches arose from research that was initiated to identify and characterize concerns about environmental contamination of locally harvested seafood and the possible human health concerns associated with consumption and use of this seafood. A consistent message from this research has been that the traditional approaches used for risk assessment of contaminated seafood fail to reflect impacts for Native American communities. This research project evaluated heavy metals, organotins, PCBs (aroclors and WHO listed congeners), chlorinated pesticides, polyaromatic hydrocarbons, and dioxins/-furans contamination in two species of clams and in Dungeness crab. Human health risk estimates were compared with tribal data analysis. Current methods were identified, data gaps and uncertainties and limitations were identified for the traditional risk assessment approaches. Seafood consumption estimates were developed using conventional fish consumption survey tools and were compared with data collected from "seafood diet interviews". This method was created by the Swinomish Tribal Community as an alternative method for evaluating the "market basket survey" within a tribal cultural context using both statistical and ethnographic methods. This latter consideration was developed in order to evaluate the currently suppressed ingestion rate data. The recognition of culturally different diets is important to accurately describe risks and protect tribal members. This presentation will include: the use of traditional harvesting techniques in sampling procedures, culturally competent mitigation options, and selection of culturally appropriate outreach and education efforts.

IS 603 BALANCING THE HEALTH BENEFITS AND RISKS OF SEAFOOD CONSUMPTION.

D. Bellinger. *Neurology-Children's Hospital, Harvard Medical School, Boston, MA.* Sponsor: E. Faustman.

Most evaluations of the health impacts of seafood consumption have focused on either benefits or risks but rarely both. This has resulted in confusing guidance to consumers and increased challenges to risk assessors. Some recent studies focusing on methyl mercury have attempted to estimate the associations between fish nutrients and contaminants and health outcomes within the same analyses. The health outcomes on which these studies focused were children's neurodevelopment (e.g., Project Viva, the Faroes Islands, the Seychelles Islands studies) and adult cardiovascular disease (e.g., the Kuopio Ischaemic Heart Disease Risk Factor Study in Finland). In all of these studies, the associations between methyl mercury and health status became more strongly inverse when fish consumption or estimates of the intake of long chain polyunsaturated fatty acids (LCPUFAs) were included in the regression models. Similarly, the associations between fish consumption or LCPUFA intake and health status became more strongly positive when biomarkers of methyl mercury levels were considered. These studies suggest that unless both the benefits and risks of seafood consumption are considered jointly in analyses, both will be underestimated. This presentation will review these studies and their implications of the findings for risk assessment and for the formulation of consumption advisories to consumers.

604 INNATE CONTROL OF ADAPTIVE IMMUNITY: DENDRITIC CELLS AND TCDD.

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TCDD is an environmental pollutant that suppresses immune function. Dendritic cells (DCs) are antigen presenting cells involved in innate and adaptive immunity. Recent studies have demonstrated that TCDD can affect the differentiation of murine bone marrow-derived dendritic cells (BMDCs). However, the effects of TCDD on the innate function of BMDCs have not been defined. We hypothesize that TCDD induces maturation of BMDCs and alters their innate immune function. To test this hypothesis, immature, GM-CSF-derived BMDCs from C57Bl/6 mice were generated in vehicle or 10 nM TCDD and assessed phenotypically and functionally. Immature DCs grown in TCDD displayed increased levels of CD11c, MHC Class II and CD86 when compared to vehicle-treated samples, indicating enhanced maturation of the DCs. Following stimulation of BMDCs with TLR ligands including CpG (TLR9), LPS (TLR4) and imiquimod (TLR7), TCDD enhanced the innate responsiveness of the BMDCs. Expression of CD86 and MHC Class II was upregulated on BMDCs grown in 10 nM TCDD and subsequently activated with TLR ligands. TCDD decreased the production of IL-10 by BMDCs stimulated with CpG but not LPS or imiquimod. In contrast, IL-12p70 production by TCDD-treated BMDCs was increased following activation with CpG and imiquimod but decreased after LPS exposure. In separate experiments, BMDC uptake of acetylated LDL and ovalbumin but not latex beads was decreased following TCDD exposure. Taken together, these results suggest that TCDD induces maturation of BMDCs, ultimately resulting in an aberrant hyperresponsiveness to TLR stimulation and a decreased ability to internalize soluble antigens. Thus, TCDD alters the innate function of DCs which ultimately may also affect the generation of adaptive immunity. This research was supported by grants from NIEHS (R01ES013784) and NCRR (P20RR17670).

605 MECHANISMS OF ETHANOL MEDIATED SUPPRESSION OF MACROPHAGE ACTIVATION.

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Previous studies indicate that ethanol acts, at least in part, to alter LPS-induced cytokine production by macrophages by preventing TLR4 receptor complex formation and/or receptor clustering. This apparently involves the inhibition of the movement of CD14 to lipid rafts, where it forms an essential part of the TLR4 receptor complex. If this is correct, it would be expected that a toll-like receptor that does not require CD14 or form receptor clusters (such as TLR3) would not be affected in the same way as TLR4 by ethanol and other agents. The production of TNF-alpha by the mouse macrophage-like cell line, RAW 264.7, was used to indicate activation of these cells. The TLR4 stimulus, LPS, and the TLR3 stimulus, poly I:C were evaluated. The effects of ethanol and of a drug used in Europe to treat withdrawal symptoms in alcohol dependent persons, chlormethiazole, on cytokine production were tested. Ethanol inhibited TNF-alpha production induced by either LPS or poly I:C. In contrast chlormethiazole caused an increase in TNF-alpha

production in cells stimulated with LPS (with a peak effect at 16 μg/ml) and poly I:C (with increases from 1-50 μg/ml), although this increase in poly I:C treated cells was not quite significant. This general pattern of results was consistent with observations using confocal microscopy. No TLR4-CD14 clustering or co-localization was observed in untreated cells or cells treated with LPS (100 ng/ml) plus ethanol (0.4%, w/v). However, clustering and co-localization were observed in cells treated with LPS alone. These results suggest that even though chlormethiazole causes similar effects as ethanol in the central nervous system, it does not act by the same mechanism as ethanol on macrophages. It remains unclear why ethanol inhibits both LPS and poly I:C induced macrophage activation. It is possible that ethanol acts on the initial signaling events and later events in TLR4 signaling but only on the later events in TLR3 signaling. This work was supported by NIAAA grant AA009505.

606 AHR ACTIVATION REDUCES DENDRITIC CELL FUNCTION BUT NOT MIGRATION IN INFLUENZA VIRUS-INFECTED MICE.

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The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that is expressed in cells of the immune system. The pollutant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is the most potent AhR agonist characterized, and is a well known immune suppressant. We have previously shown that AhR activation by TCDD suppresses the proliferation and differentiation of influenza virus-specific CD8+ T cells in the mediastinal lymph node (MLN). However, TCDD does not affect CD8+ T cells directly, suggesting that defects in accessory cells such as antigen presenting cells (APCs) or regulatory CD4+ T cells (Treg) are affected by AhR activation. In this study, we show that the suppression of influenza virus-specific CD8+ T cell response by TCDD appears to be independent of CD4+ cells. This finding suggests that deregulated APC function, not Treg function, underlies the TCDD-mediated decrease in response of virus-specific CD8+ T cells. Dendritic cells (DC) are highly specialized APCs that have a unique ability to induce and direct Ag-specific responses in naïve T cells. For example, respiratory DC sample viral antigen and migrate to the MLN, where they stimulate clonal expansion and differentiation of virus specific CD8+ T cells, which are ultimately recruited to the lung to kill infected host cells. Using CFSE to label respiratory DC and track their migration, we show that AhR activation does not affect the migration of respiratory DC to the MLN in response to virus infection. However, DC isolated from the MLN of virus-infected mice significantly reduced the proliferation of naïve virus-specific CD8+ T cells. These results suggest that AhR activation by TCDD impairs DC function, which leads to the impaired CD8+ T cell response to influenza virus.
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607 ACTIVATION AND TRAFFICKING OF PERITONEAL B1A B CELLS IN RESPONSE TO ASBESTOS.

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Exposure to inhaled silicates, including crystalline silica and asbestos, is associated with development of autoantibodies and systemic autoimmunity in both mice and humans, but the etiology of the autoimmune responses remains unclear. C57Bl/6 mice have been shown to develop autoimmune pathology following exposure to amphibole asbestos, with accumulation of the B1a subset of B lymphocytes in peripheral lymph nodes. B1a cells are implicated in the production of autoantibodies, and in some mouse models B1a cell depletion ameliorates the symptoms of autoimmune pathologies. B1a B cells are found primarily in the peritoneal and pleural cavities but traffic to the spleen or sites of inflammation where they are major producers of T-independent IgM, some of which is auto-reactive. However, the mechanism of trafficking and their role in silicate-induced autoimmunity are not known. To test the hypothesis that asbestos would cause activation and trafficking of peritoneal B1a cells, C57Bl/6 mice were given 100 μg of amphibole asbestos, or saline alone for controls, via intra-peritoneal injection. Peritoneal cells were obtained by lavage of the intact peritoneal cavities with warm media. Intraperitoneal adoptive transfer of congenic CD45.1 C57Bl/6 peritoneal cells allowed analysis of B1a cell trafficking to the spleen following asbestos exposure. Multi-color flow cytometry demonstrated a decline in the number of congenic B1a B cells in the peritoneal cavity with a concurrent increase in the spleen over six days. The loss of B1a cells from the peritoneum was not due to cell death. By three days after treatment, the percentage of B1a cells expressing CD69 (early activation marker) increased with asbestos treatment, while alpha-4 integrin expression was significantly reduced on those cells, suggesting preparation for trafficking. We conclude that, in C57Bl/6 mice, peritoneal B1a B lymphocytes become activated and traffic to the spleen following exposure to asbestos. This work was funded by NCRR P20 RR017670-06.

608 EFFECTS OF THE HEAVY METALS MERCURY AND LEAD ON IL-33-INDUCED MAST CELL CYTOKINE SECRETION.

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Interleukin-33 (IL-33) is an IL-1 cytokine family member and is a potent activator of NF-κB and consequently the synthesis and secretion of the cytokines IL-6 and IL-13 by human and murine mast cells. Recently, we have shown that IL-33 is secreted at high levels from central nervous system (CNS) glia during inflammatory conditions. The heavy metals mercury (Hg) and lead (Pb) are known to accumulate in the CNS in exposed individuals. Thus, in Hg/Pb exposed individuals, both IL-33 and Hg/Pb would be present in the CNS after CNS infection/injury and these factors could act on mast cells, a cell type present in great quantity in/around the CNS. To determine the effects of Hg and Pb have on cytokine secretion induced by IL-33 and other NF-κB inducers, the murine mast cell line MC/9 was pre-incubated with either Hg or Pb for 24 hours and then stimulated with either IL-33, LPS, PAM3Cys or double stranded RNA (dsRNA). As expected, all 4 inducers of NF-κB induced the secretion of IL-6, IL-13 and MCP-1 with IL-33 being the strongest stimulus. Surprisingly, Hg and Pb (Hg >> Pb) significantly increased IL-6 secretion in both unstimulated and stimulated cultures. This increase in IL-6 was not observed for IL-13 or MCP-1 as Hg inhibited IL-13 and MCP-1 secretion in stimulated cultures, while Pb had no significant effects other than to increase MCP-1 secretion from unstimulated cultures. Interestingly, Hg also significantly induced TGF-β expression, while Pb could not. Thus, it appears that Hg, but not Pb, is able to skew IL-33 (NF-κB) driven mast cell cytokine secretion towards the production of Th17-skewing cytokines (IL-6 and TGF-β) and away from cytokines important in Th2 responses (IL-13 and MCP-1). As both Hg and Th17 have been strongly implicated in organ-directed autoimmunity, it is to tempting to speculate that the effects of Hg on mast cells play a role in the mechanism of the initiation of Hg-induced autoimmunity.

609 CONTRIBUTION OF P53 AND BCL-2 FAMILY MEMBERS IN PROSTAGLANDIN AND PHTHALATE-INDUCED PRO/PRE-B CELL APOPTOSIS.

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Phthalate esters are ubiquitous environmental contaminants that are produced for a variety of common industrial and commercial purposes. We have shown previously that mono-(2-ethylhexyl) phthalate (MEHP), the toxic metabolite of di-(2-ethylhexyl) phthalate, the main plasticizer used in polyvinyl chloride, induces apoptosis in bone marrow B cells and that this apoptosis is enhanced in the presence of the endogenous prostaglandin 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2). MEHP and 15d-PGJ_2 induced significant apoptosis within 8 and 5 hrs, respectively, in cultured pro/pre-B (BU-11) cells and acted cooperatively to induce apoptosis in primary pro-B cells. Apoptosis was accompanied by the activation of multiple initiator caspases and executed by caspase-3. MEHP- and 15d-PGJ_2-induced apoptosis were reduced in APAF1 null primary pro-B cells, indicating an intrinsically activated pathway. Apoptosis was accompanied by loss of mitochondrial membrane potential and release of cytochrome c. A significant translocation of Bax to the mitochondria supports its role in initiating release of cytochrome c. Cleaved Bid was observed following treatment with either chemical; however, a greater portion of the total Bid pool was cleaved following treatment with 15d-PGJ_2, potentially differentiating the two pathways. Co-treatment with Z-VAD-FMK significantly reduced 15d-PGJ_2-induced Bid cleavage, which was correlated with reduced release of cytochrome c. Bid cleavage and subsequent release of cytochrome c most likely occurs as a secondary mechanism to amplify the apoptotic signal following a small, but essential, caspase-independent release of cytochrome c that acts as the apical event in chemical stress-induced apoptosis in early B cells. Initial results suggest that p53 plays an integral role in the initiation of the intrinsic pathway by MEHP and 15d-PGJ_2. Elucidation of these pathways will increase our understanding of how chemical mixtures may enhance the susceptibility of pro/pre-B cells to environmental contaminant-induced apoptosis.

610 BENZO(A)PYRENE AND 7, 12-DIMETHYLBENZ(A)ANTHRACENE DIFFERENTIALLY DISRUPT HAEMATOPOIESIS IN BONE MARROW AND LYMPHOID ORGANS.

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Benzo(a)pyrene (BP) and 7,12-dimethylbenz(a)anthracene (DMBA) are carcinogenic PAHs that selectively deplete mouse bone marrow (BM) cells. This process is dependent on Cyp1b1 but surprisingly suppressed by AhR activation. We have pre-

viously shown that, in C57BL/6J mice, total BM cellularity decreased 2-fold following ip DMBA but not BP treatment. This investigation shows that the DMBA mediated decrease in BM cells targets early progenitor cells, and does not affect mature BM cell numbers. Wild type female C57BL/6 mice were injected ip with 10 or 50 mg/kg DMBA or BP, and the effect on BM lymphoid and myeloid progenitors determined over time. DMBA treated animals exhibited dose-dependent decreases in BM cells and disruption of marrow structure at 24 to 168 h post treatment. Flow cytometric analysis of BM cells revealed significant decreases in lymphoid and myeloid populations, consistent with the decrease in BM cells following DMBA treatment. BM cells showed significant dose-and time-dependent decreases in progenitor lymphoid (CFU-preB) and myeloid (CFU-GM) cells following DMBA treatment. In contrast, BP treated mice showed minimal effects. In addition, DMBA caused a greater decrease in spleen, thymus, and white blood cell counts. Changes in lung and liver weights were minimal, consistent with the IP administration that bypasses liver metabolism by Cyp1a1. Given that BP and DMBA are both metabolized by Cyp1b1 in the BM, the differential effects on BM cells may stem from differences in AhR-mediated removal of their reactive metabolites. Congenic mice with the low affinity AhR (AhRd), when treated with 50 mg/kg of BP or DMBA displayed similar toxicity for BM cells. This observation indicates AhR involvement in the selective effects of PAHs on BM haematopoiesis, in vivo, which slow the developmental program from haematopoietic stem cells through progenitor cells that subsequently mature and populate the spleen and lymphoid tissues.

611 ORGANIC CATION TRANSPORTERS 1 AND 2 MEDIATE PRALIDOXIME RENAL SECRETION.

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Objective: Pralidoxime (PRX) is an organic cation used as an antidote to treat organophosphate poisoning. The elimination of PRX is characterized by fast renal excretion considered the main determinant of PRX pharmacokinetics. In rodents, we studied the role of organic cation transporters (OCT) in the renal secretion of PRX using specific OCT substrates (TEA) and Knock-out mice (OCT1/2-/-; OCT3-/-). Methods: Male Sprague-Dawley rats received a loading dose of PRX (31 mg/Kg) followed by a continuous infusion to achieve a plasma PRX concentration of 10 mg/l during 180 min. Then, blood and urine were collected. In rats, the pharmacokinetics of PRX (50 mg/Kg, 30 min perfusion) was determined 15 min after TEA treatment (75 mg/kg, IM). Knock-out and wild type mice received a PRX injection (50 mg/Kg, IM), blood samples were taken at 45 and 90 min post-injection. Results are expressed as mean ± SEM. Statistical analysis used t-test and 2 way analysis of variance on log transformed data with $p< 0.05$. Results: Renal clearance of PRX (30.1±1.8 ml/min/kg) was significantly higher ($p< 0.001$) than t creatinine clearance (9.6±0.9 ml/min/kg) evidencing the renal secretion of PRX. Pretreatment by TEA significantly modifies the pharmacokinetics of PRX, increasing the beta half-life (224 vs 39 min-1) and decreasing the clearance of PRX(3.6 vs 2.2 l/h/kg) without modification of the volume of distribution. The defiency in organic cation transporters 1 and 2 in mice (OCT1/2-/-) resulted in a significant increase ($p< 0.001$) in plasma pralidoxime concentrations at 45 min (6.40±0.46 vs 2.37±0.13 mg/l) and 90 min (2.83±0.17 vs 1.47±0.07). Lack in OCT3 did not change the plasma PRX concentrations. Conclusion: Our data show that PRX is secreted in urine by an active process involving organic cation transporters 1 and 2 (but not 3).

612 PBPK MODELING OF DELTAMETHRIN IN RATS.

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The pyrethroid pesticide deltamethrin is cleared nearly twice as rapidly in human liver microsomes compared to rat liver microsomes. A species difference such as this could influence the toxic potency of deltamethrin between rats and humans. PBPK modeling is a tool that can be utilized to examine the impact that such a species difference may have on exposure-dose relationships. A previous PBPK model for deltamethrin in the rat by Mirfazaelian et al. (Toxicol Sci. 2006 Oct;93(2):432-42) suggests that absorption is dose dependent with little absorption of environmentally relevant exposures. In addition, the model employs both flow and diffusion-limited compartments and splits the blood compartment into plasma and red blood cells. Oral bioavailability studies were designed to examine the dose dependency in absorption. The results of theses studies indicate there was no significant difference in the fraction absorbed of oral doses of 0.3 and 3.0 mg deltamethrin/Kg. In contrast to the previous model which utilized both flow- and diffusion-limited tissue compartments the current model described all tissue compartments with diffusion-limited kinetics. The present model also describes the blood as a single compartment. These changes resulted in an improved ability of the deltamethrin PBPK model to describe the shape of the tissue concentration-time curves for both literature data and data from the present oral bioavailability studies. The description of the liver by diffusion- limited kinetics has the effect of reducing the impact of the species difference in metabolism since diffusion is the rate limiting step in the metabolic elimination of deltamethrin. (This abstract does not represent EPA policy; S.J.G. was supported by NHEERL-DESE, EPA CT826513 and, in part, by a NIEHS Training Grant T32-ES07126).

613 EFFECT OF NICOTINE EXPOSURE ON IN VITRO CYP450S-MEDIATED METABOLISM OF CHLORPYRIFOS (CPF) IN SPRAGUE-DAWLEY (S-D) RATS.

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Chlorpyrifos (CPF) is a common organophosphate (OP) insecticide which is metabolized by CYP450s to the neurotoxic metabolite chlorpyrifos-oxon (CPF-oxon) and a non-toxic metabolite 3,5,6-trichloro-2-pyridinol (TCP). The objective of this study was to quantify the *in vivo* effect of repeated nicotine exposure on *in vitro* metabolism of CPF and marker substrates in rats. Male S-D rats (~ 275 g bw) were divided into 6 groups (n = 4) and dosed s.c. with 1 mg nicotine/kg/day in saline, controls were given saline only. Animals showed signs of cholinergic crisis after the initial nicotine doses, but exhibited adaptation after a couple days of treatment. Rats were sacrificed after 4 hr, 24 hr, 7 days, 7 days + 4 hr, or 10 days of nicotine-treatment, and hepatic microsomes were prepared and stored at -80°C until analysis. While CYP450 reduced CO spectra were not different across the treatments, the 24 hr-group showed a 2-fold increase in CYP2E1 marker substrate (p-nitrophenol: PNP) activity over controls, whereas longer treatments resulted in no difference in PNP hydroxylase activity. The 7 day + 4 hr- and 10 day-groups showed decreased EROD/PROD marker substrate activity. Moreover, in 7 day- and 10 day-nicotine-treated groups, the estimated pesudo-1st order clearance rate constants (Vmax/Km) for TCP exhibited a 2-fold increase over that of controls, suggesting possible induction of CYP-mediated metabolism of CPF to TCP. The results of this *in vitro* study suggest that repeated nicotine exposure (i.e., from smoking) could alter the metabolism of OP pesticides. Future *in vivo* experiments based on these results will be conducted to ascertain the impact of *in vivo* nicotine exposures on CPF metabolism in rats. (This work was supported by NIOSH/CDC R01-OH003629-04)

614 DISPOSITION OF NOVEL NANOPARTICLE CONSTRUCTS IN JUVENILE SWINE.

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Gold and Palladium nanoparticles have many potential uses in medicine, including the enhancement of computed tomography (CT) imaging and targeted cancer therapy and detection. Several experiments have been conducted with the objective of determining the disposition of palladium and gold nanoparticles (NPs) in blood and tissues following intravenous and inhalation delivery to juvenile swine. Juvenile swine were anesthetized with isoflurane, and venous catheters were placed in the cranial vena cava. The nanoparticles were administered via the catheter at 2 mg Au or Pd/kg BW. Serial blood samples were collected at 15, 30, 60, 120, and 240 minutes post-dosing. A final blood sample was then collected at 24 hours and pigs were humanely sacrificed and tissues were collected for gold or palladium analysis using atomic absorption spectrophotometry or inductively coupled spectrometry. One of the gold nanoparticle compounds was delivered by the inhalation route via the anesthetic circuit to compare pharmacokinetic parameters to the same nanoparticle construct given intravenously. Nanoparticles were distributed according to a two-compartment model. There were measurable levels of gold in most tissues, however, the lung, liver, spleen, and kidney had the greatest concentrations. Retention of gold NPs administered by the IV route is long-lived with more than 90% of the dose retained in liver, lung, and spleen 90 days following an IV dose of 2 mg Au/kg body weight. Supported by the National Institutes of Health/National Cancer Institute, Cancer Nanotechnology Platform program (grant number: 1R01CA119412-01).

615 THE ORGANIC SOLUTE TRANSPORTER ALPHA AND BETA, A POTENTIAL STEROID HORMONE CONJUGATE TRANSPORTER IN STEROIDOGENIC TISSUES.

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The organic solute transporter alpha and beta (Ostα-Ostβ) is a heteromeric transporter that is expressed in most tissues, but it is most abundant in testis, adrenal gland, intestine, liver and kidney. In the intestine it is localized to the basolateral membrane of enterocytes where it functions in the reabsorption of bile acids and perhaps structurally-related molecules of both endogenous and exogenous origin. The present study tested the hypothesis that Ostα-Ostβ is a steroid hormone conjugate transporter in steroidogenic tissues, and thus that it may play an important role in regulating steroid hormone homeostasis. Substrate specificity of Ostα-Ostβ was examined in Xenopus laevis oocytes that had been microinjected with Ostα and Ostβ cRNA. A wide range of steroid hormone conjugates cis-inhibited Ostα-Ostβ-mediated uptake of a known substrate, 3H-estrone sulfate, whereas the parent steroid hormones had no effect. Selected steroid hormone conjugates also trans-stimulated Ostα-Ostβ-mediated efflux of 3H-estrone sulfate. These results suggest that steroid hormones conjugates are substrates of this transporter, but the parent compounds are not. Additional studies revealed that pregnenolone sulfate is also a substrate for Ostα-Ostβ. Pregnenolone sulfate and dehydroepiandrosterone sulfate (DHEAS), another known substrate for Ostα-Ostβ, are active steroid hormone precursors which circulate in the human body with a relatively high concentration, and can be converted to active steroid hormones in target tissues. Because there is increasing evidence that these hormones are involved in the pathogenesis of breast and prostate cancer, characterizing the expression level and localization of Ostα-Ostβ in steroidogenic tissues may provide insight into the etiology of these diseases. (Supported in part by NIH grants DK067214, ES01247, and ES07026)

616 UPTAKE OF PERFLUOROOCTANOIC ACID BY FRESHLY ISOLATED HEPATOCYTES FROM MALE AND FEMALE RATS.

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Perfluorooctanoic acid (PFOA) is a commercially important fluorochemical. The renal elimination of PFOA in rats is sex-dependent, being much faster in female rats than that in the males. Liver is a primary target organ for PFOA distribution in rat. Administration of PFOA to rats results in peroxisome proliferation and benign liver tumors, via the activation of peroxisome proliferator-activated receptors. In this study, we investigated the hepatic uptake of PFOA using freshly isolated hepatocytes from male and female rats. We identified a non-specific and non-saturable cell partitioning process for PFOA at low-temperature (on-ice) incubation conditions. At 37 °C, hepatocyte uptake of PFOA was composed of the non-specific partition as well as a saturable, active uptake process. The K_m and V_{max} values for the active uptake process were determined to be 88.0 ± 9.1 μM and 5.61 ± 0.88 nmol/min/10^6 cells, respectively, for male rat hepatocytes, and 76.1 ± 12.0 μM and 3.59 ± 0.29 nmol/min/10^6 cells, respectively, for female rat hepatocytes. The values of PFOA clearance by active uptake (defined by V_{max}/K_m) were 64.8 ± 15.7 μL/min/10^6 cells for male rat hepatocytes and 47.6 ± 4.7 μL/min/10^6 cells for female rat hepatocytes. The active uptake of PFOA in rat hepatocytes was inhibited by known substrates of organic anion transporters (Oats) and organic anion transporting polypeptides (Oatps). Inhibition of PFOA uptake by sulfobromophthalein (BSP) was further characterized and values of the apparent inhibition constant, $K_{i,app}$, were obtained for both male and female rat hepatocytes.

617 EFFECT OF NRF2 ACTIVATION ON BILIARY EXCRETION OF BSP AND DBSP.

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Sulfobromophthalein (BSP) is a prototypical drug for studying hepatobiliary function. BSP is conjugated with glutathione (GSH), whereas its dibrominated analog disulfobromophthalein (DBSP) is not conjugated with GSH before biliary excretion. Nuclear factor-E2-related factor-2 (Nrf2) is a transcription factor that can induce cytoprotective genes. After oxidative stress, Nrf2 dissociates from its cytosolic repressor Keap1 and increases the mRNA expression of GSH synthetic enzymes, GSH-*S*-transferases (Gsts), and Mrp transporters. Mrp2 transports GSH-conjugates into bile, whereas Mrp3 transports GSH-conjugates into blood. The purpose of this study was to determine whether an increase in Nrf2 activation enhances the elimination of BSP and DBSP, and if so, whether it is due to altered biotransformation or transport. To test this hypothesis male wild-type (WT), Nrf2-null and Nrf2 enhanced (EN)–(Keap1-deficient mice) were injected intra-arterially with BSP or DBSP. Bile and blood samples were collected for 1hr. The biliary excretion of BSP was significantly different in these mouse genotypes. Within 30 min, Nrf2-null mice excreted 25%, WT mice 52%, and Nrf2-EN mice 80% of injected BSP. Liver GSH content was not altered by biliary excretion of BSP. In all three genotypes of mice, the biliary excretion of GSH and the gene expression of major Gsts were proportional to the amount of Nrf2. BSP-GSH conjugation activity in liver cytosol of Nrf2-null and Nrf2-EN mice was 42% and 554% of WT mice, respectively. There were no differences in biliary excretion or plasma disappearance of DBSP in the three genotypes. Because the same carriers transport BSP and DBSP, and BSP does not decrease the hepatic GSH concentration, the enhanced rate of biliary excretion of BSP appears to be due to the induction of Gsts that conjugate BSP. The results show that Nrf2 can increase the biliary excretion of some chemicals by increasing their conjugation with GSH by Gsts. (Supported by NIH grants ES09649, ES09716, ES013714, and ES07079.)

618 TISSUE DISTRIBUTION OF THE MACROCYCLIC TRICHOTHECENE SATRATOXIN G FOLLOWING INTRANASAL INSTILLATION IN THE MOUSE.

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Inhalation of aerosolized fungi and its components in the environment is a growing health concern, especially in damp buildings. Trichothecene mycotoxins, which are sesquiterpenoid fungal metabolites, can evoke systemic and airway toxicity when inhaled. Although satratoxin G (SG), a macrocyclic trichothecene produced by Stachybotrys chartarum (black mold), was recently found to induce olfactory sensory neuron (OSN) apoptosis, rhinitis and focal encephalitis in mice, relatively little is known about SG tissue distribution following inhalation exposure. The purpose of this study was to test the hypothesis that SG is preferentially retained in the nasal compartment following intranasal exposure. Female C57BL/6 mice were intranasally instilled with SG (250 μg/kg bw). The mice were euthanized at various time intervals and tissues collected for SG analysis by a competitive direct enzyme-linked immunosorbent assay (ELISA). SG was rapidly (15 min) and differentially distributed among the tissues analyzed. Nasal turbinates had the highest SG peak concentration (477 ng/g tissue), whereas spleen, lung and kidney ranged between 196-280 ng/g tissue at peak. Heart, thymus and liver had peak SG concentrations ranging from 69-105 ng/g tissue, while much lower maximal concentrations (<10 ng/g tissue) were detectable in plasma, brain and olfactory bulb. Most tissues assayed had SG elimination half lives (T1/2) of < 4.5 h. However, the nasal turbinates, olfactory bulb and brain had T1/2 of > 7.5 h suggesting a slower clearance. Taken together, these data suggest that the high concentrations and slow clearance observed in nasal turbinates following intranasal delivery of SG was consistent with OSN apoptotosis and rhinitis previously observed. The capacity of SG to differentially distribute and slowly clear from several other organs might contribute to other toxic sequelae associated with exposure to Stachybotrys and its macrocyclic trichothecenes.

619 DEVELOPMENT OF PREDICTIVE *IN VITRO* ASSAYS FOR THE DETECTION OF STEATOSIS IN PRIMARY RAT HEPATOCYTES.

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Therapeutic agents that cause abnormal neutral lipid accumulation in the liver (hepatosteatosis) can lead to drug-induced liver injury. Early in vitro identification of compounds that cause steatosis will help reduce compound attrition. Here we describe the use of lipophilic fluorescent markers in the identification of stored neutral fat within primary rat hepatocytes using imaging technology. Bespoke algorithms were designed to quantify differences in the localisation and morphology of lipid droplets in hepatocytes. Incubation of hepatocytes under different hormonal and nutritional states altered lipid metabolism. Under conditions that promote lipid uptake and oxidation, a decrease in the staining of the fluorescent lipid analogue, BODIPY 493/503 was observed. Specificity of the dye for neutral lipid droplets was confirmed by the presence of an adipophillin "coat" around BODIPY stained droplets. Under these conditions a larger signal window was observed following treatment of steatotic compounds (e.g. amiodarone) compared to conditions promoting glucose catabolism. A panel of steatotic compounds including amiodarone, ibuprofen and methotrexate produced a dose dependent increase in the amount of neutral lipid stored in the cell. Subsequent co-localisation studies using Nile Red (a lipophillic dye) and BODIPY FL C16 (fluorescently labelled lipid) revealed subpopulations of lipid droplets: complete co-localisation of Nile Red and BODIPY was observed in amiodarone treated cells but not in ibuprofen treated cells. Defects in mitochondrial b-oxidation have been cited as one of the

mechanisms leading to drug-induced steatosis. We used tetramethyl-rhodamine ethylester to monitor mitochondrial membrane potential in compound treated rat hepatocytes. We observed morphologic and functional changes in mitochondria with steatotic compounds. The different lipid and mitochondrial phenotypes may reflect the causative mechanisms of drug-induced steatosis. Collectively, these in vitro assays allow the identification of steatotic compounds.

620 USE OF RAT AND HUMAN SANDWICH-CULTURED HEPATOCYTES AND A CASSETTE DOSING APPROACH TO ASSESS INHIBITION OF BILE ACID TRANSPORT.

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Inhibition of hepatic bile acid (BA) transport may be one mechanism of drug-induced liver injury. An *in vitro* hepatic model with functional metabolic and transport systems would be useful to identify compounds that inhibit hepatic uptake and/or excretion of BAs. The aim of this study was to determine whether known hepatotoxic drugs inhibit BA transport using a cassette dosing (CD) approach in combination with sandwich-cultured rat (SCRH) and human (SCHH) hepatocytes. A set of drugs (n=28) known to elicit hepatotoxicity was selected. The effect of these drugs (individually or in combination) on the biliary excretion index (BEI; a relative measure of the extent of biliary excretion) and *in vitro* biliary clearance (Cl_{bile}) of [^{3}H]-taurocholate (TC), an NTCP/BSEP probe substrate, was determined in SCRH and SCHH at clinically relevant concentrations; effects were expressed as a percentage of vehicle control data. In control SCRH, TC accumulation (cell+bile), BEI, and Cl_{bile} were 36.7±5.8 pmol/mg protein, 87.5%±1.9, and 26.0±4.6 mL/min/kg, respectively (n=6 livers, in at least duplicate; mean±SEM). Cyclosporin A, troglitazone, and rifampicin decreased TC BEI (25, 20, and 67%, respectively) and Cl_{bile} (37, 87, and 91%, respectively), suggesting that these drugs primarily inhibited BSEP. In contrast, azlocillin, cloxacillin, carbenicillin, oxacillin, pioglitazone, amiodarone, nafcillin, and ampicillin decreased Cl_{bile} (20% to 43%) with no notable change (<20%) in BEI. Inhibition of Cl_{bile} but not BEI suggests that these drugs primarily inhibited BA uptake. Subsequent studies using a CD approach (n=2-4 compounds per cassette; 60 cassettes total) in SCRH yielded similar results. In addition, CD in SCHH yielded results consistent with those observed for SCRH. In conclusion, a CD approach in combination with SCH is a useful *in vitro* system to identify compounds that inhibit BA uptake and/or excretion. *Supported in part by NIH GM41935, NIH CA106101, and GSK.*

621 MICROFLUIDIC ARRAY FOR HIGH THROUGHPUT PRIMARY HEPATOCYTE CULTURE AND ANALYSIS.

P. J. Lee and P. J. Hung. *CellASIC Corp., San Leandro, CA.* Sponsor: P. Help.

Primary hepatocytes represent a sophisticated in vitro model for drug toxicity screening. However, current methods are unable to maintain functional liver-specific activity, and are further limited by the high cost of cell samples. We have developed a microfluidic culture format that addresses these two pain points by: 1) engineering a more controlled microenvironment in order to recreate liver-specific hepatocyte functions in culture, and 2) generating an arrayed format compatible with existing high throughput methods that utilizes 1/10th the cells of typical screening tools. Each microfluidic culture unit consists of a microfabricated bioreactor designed to maintain hepatocytes in a high density sinusoid-like mass that preserves cell-cell contacts while exposing cells to a continuous flow of solution through a microfabricated channel network. An innovative porous endothelial-like barrier localizes cells into 100 micron wide sinusoids while facilitating mass transport to/from the cells. The channels also support collagen gel culture, demonstrating the ability to maintain albumin production, basal P450 activity, and cell viability for over 7 days. The microfluidic bioreactors are formatted to fit on a standard 384 well dish and operate with standard robotic platforms. Each 384 well plate contains 128 culture units (3 wells for: inlet, outlet, and cell culture). The fluidics of the device are designed to operate using gravity and surface tension forces such that no external machinery (besides a automated liquid dispenser) is necessary to perform hepatocyte culture and analysis. A glass bottom surface gives superior optical quality for microscopy, high content imaging, and plate readers. Each well can be loaded with 5 microliters of cell suspension, providing improved throughput and reduced cost for primary hepatocyte screening.

622 TOXICOGENOMIC ASSESSMENT OF SPECIES- AND SEX-DEPENDENT MECHANISMS OF HEPATOCARCINOGENICITY USING 3-D LIVER TISSUE CO-CULTURES.

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Human and rodent 3-D liver tissue co-cultures were exposed to hepatocarcinogens to determine their ability to model known species- and sex-dependent mechanisms of hepatocarcinogenicity reported in the literature from in vivo rodent studies and human exposure data. Parenchymal (hepatocyte) and microenvironmental (ME) cells isolated from human or rodent livers were co-cultured on 3-D interconnecting porous scaffolds in multiwell plates to recreate functional liver tissue in vitro for high throughput screening. Co-culture with ME cells such as Kupffer cells, fat cells, endothelia, (e.g. all the cells of the native liver) supports a broader range of functionality of hepatocytes in the 3-D tissues and enhances the functional longevity of the co-cultures. Cell viability, liver-specific protein, genomic, drug metabolism and cytochrome P450 (CYP450) data demonstrate the sustained liver tissue function of these 3-D co-cultures for weeks to months in static multiwell plates. The 3-D liver tissue co-cultures were exposed to various toxins, including non-genotoxic carcinogens such as Phenobarbital (PB). Tritiated thymidine incorporation into DNA of 3-D liver co-cultures was stimulated by PB treatment and increased dramatically with exposure time over weeks in vitro. Quantitative PCR of various CYP450 genes as well as some well known tumor suppressor and protooncogenes were analyzed and compared to gene chip data. Non-genotoxic carcinogens require sustained/chronic exposure to elicit their effects. The long-term sustained function of the in vitro 3-D liver tissue co-cultures makes them an ideal model system for assessment of the effects of chronic drugs such as PB. Exposure of 3-D liver tissue co-cultures grown from male and female rat, mouse and human donors to PB and other hepatocarcinogens will be presented. Data will be compared to in vivo results as well as short-term studies in hepatocyte monolayer cultures.

623 TROVAFLOXACIN ENHANCES THE INFLAMMATORY RESPONSE TO A GRAM-NEGATIVE OR A GRAM-POSITIVE STIMULUS, RESULTING IN NEUTROPHIL-DEPENDENT LIVER INJURY IN MICE.

P. J. Shaw, P. E. Ganey and R. A. Roth. *Pharmacology & Toxicology, Michigan State University, East Lansing, MI.*

Trovafloxacin (TVX), a fluoroquinolone antibiotic, has a broader spectrum of activity than other quinolones against gram-positive bacteria while maintaining its gram-negative bacteriacidal activity. In 1999, the use of TVX was severely restricted due to the strong association with several cases of idiosyncratic hepatotoxicity. Previous studies have shown that a modest inflammatory stress induced by lipopolysaccharide (LPS) renders nontoxic doses of TVX hepatotoxic in rodents. This study explored the interaction of TVX with a subsequent inflammatory stress induced by either a gram-negative or a gram-positive bacterial stimulus. Mice were given TVX 3 h before LPS (gram-negative) or a peptidoglycan/lipoteichoic acid (PGN-LTA) mixture isolated from S. aureus (gram-positive). TVX administration resulted in significant liver injury by 4.5 h after coexposure to either LPS or PGN-LTA. Hepatotoxicity was maximal at 15 h. Histopathology revealed that TVX/LPS-coexposure resulted in primarily midzonal hepatocellular necrosis, whereas TVX/PGN-LTA-induced necrosis was primarily centrilobular. Treatment with either LPS or PGN-LTA alone increased plasma concentrations of IL-18, IL-1β, IL-6, IL-10, KC, MCP-1, MIP-1α, VEGF, IFNγ, TNFα and MIP-2 at 4.5 h. TVX treatment before LPS significantly increased all of these cytokines/chemokines. In contrast, TVX treatment before PGN-LTA significantly increased all of them except TNFα and IFNγ. The role of neutrophils was explored based on the enhancement of several chemokines by TVX. Neutrophil activation in livers was inhibited by CD18 antiserum, which also significantly reduced liver injury from either TVX/LPS- or TVX/PGN-LTA-coexposure. In summary, TVX significantly enhanced the inflammatory response of mice to either a gram-negative or gram-positive stimulus and caused hepatotoxicity which was dependent on neutrophil activation. (Supported by NIH grant DK061315.)

624 CENTRILOBULAR INDUCTION OF MRP4 DURING ALLYL ALCOHOL TOXICITY IS NOT DEPENDENT ON KUPFFER CELL FUNCTION.

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Treatment of mice with toxic doses of the centrilobular hepatotoxicant acetaminophen (APAP) results in increased hepatic mRNA and protein levels of several members of the multidrug resistance protein (Mrp) family of transporters. Mrp4 is the most significantly up-regulated transporter by APAP and its expression is localized to centrilobular hepatocytes adjacent to damaged areas. Kupffer cell (KC) activation by APAP mediates these changes in Mrp4. The present study was performed to determine if allyl alcohol (AA), a periportal hepatotoxicant, also induces Mrp4 expression. This study also investigated whether changes in Mrp4 by AA are dependent on KC function. C57BL/6J mice received 0.1 ml clodronate liposomes to deplete KC or empty liposomes 48 hrs prior to dosing with 60 mg/kg AA, i.p. Hepatotoxicity was assessed by plasma ALT and histopathology. Hepatic transporter mRNA and protein expression were determined by bDNA signal amplification assay and Western blotting, respectively. Depletion of KC by liposomal clodronate treatment resulted in heightened susceptibility to AA toxicity. Exposure to

AA increased Mrp1, Mrp3, Mrp4, and Mrp5, while decreasing Oatp1a1, Oatp1b2, Ntcp, and Mrp6 mRNA levels. Protein analysis revealed elevated Mrp1 (3-fold), Mrp4 (9-fold), and Mrp5 (9-fold), in addition to significant reductions in Oatp1a1 and Ntcp proteins. Depletion of KC had no effect on changes in transport proteins induced by AA. Surprisingly, immunofluorescent analysis of Mrp4 showed enhanced staining in centrilobular hepatocytes and not in periportal regions where necrosis by AA is noted. These findings demonstrate that KC function is protective from AA toxicity and that the induction of Mrp4 is not in direct response to hepatic injury, as this occurs in regions of the liver distant from any AA-induced necrosis. This work was supported by NIH Grants DK069557 (JEM) and DK068039 (NJC).

625 ROLE HYPOXIA-INDUCIBLE FACTOR-1ALPHA IN THE DEVELOPMENT OF LIVER FIBROSIS.

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Liver fibrosis is a disease characterized by excessive deposition of extracellular matrix, such as collagens in liver during chronic injury. During early stages in the genesis of this disease, cells begin to synthesize and secrete profibrotic proteins that stimulate matrix production and inhibit matrix degradation. Although it is clear that these proteins are important for the development of fibrosis, what remains unknown is the mechanism by which chronic liver injury stimulates their production. In the present study, the hypothesis was tested that the hypoxia-activated transcription factor, hypoxia-inducible factor-1α (HIF-1α), is activated in the liver during chronic injury and regulates expression of profibrotic proteins that promote development of fibrosis. To investigate this hypothesis, mice were subjected to bile duct ligation (BDL), an animal model of liver fibrosis. HIF-1α protein was increased in the livers of mice subjected to BDL by 3 days after surgery. To test the hypothesis that HIF-1α is required for the development of fibrosis, wild-type and HIF-1α-deficient mice were subjected to BDL. The extent of hepatocyte injury and cholestasis was not different between wild-type and HIF-1α-deficient mice. Levels of type I collagen mRNA and protein were increased in wild-type mice by 14 days after BDL. These levels were significantly reduced in HIF-1α-deficient mice. Levels of α-smooth muscle actin mRNA and protein were increased to a greater extent in wild-type mice 14 days after BDL when compared to HIF-1α-deficient mice. Next, the levels of several profibrotic mediators were measured to elucidate the mechanism by which HIF-1α promotes liver fibrosis. Platelet-derived growth factor (PDGF)-A, PDGF-B, fibroblast growth factor-2, and plasminogen activator inhibitor-1 mRNA levels were increased in wild-type mice subjected to BDL. Levels of these mRNAs were significantly lower in HIF-1α-deficient mice. Results from these studies suggest that HIF-1α is a critical regulator of profibrotic mediator production during the development of liver fibrosis.

626 MECHANISMS OF PROTECTION AGAINST ACETAMINOPHEN HEPATOTOXICITY BY GLUTATHIONE AND N-ACETYLCYSTEINE.

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The glutathione (GSH) precursor N-acetylcysteine (NAC) is used to treat patients with acetaminophen (APAP) overdose in the clinic for up to 48h. However, the mechanism of this protection remains incompletely understood. To address this issue, fasted C3HeB/FeJ mice were treated with 300 mg/kg AAP and then received intravenously either 0.65 mmol/kg GSH, NAC or a combination of the individual amino acids of GSH (glycine, glutamic acid and cysteine) at 1.5 h after APAP. The animals were sacrificed at 6 h and liver injury (plasma ALT activities, area of necrosis), DNA fragmentation, peroxynitrite formation (nitrotyrosine staining) and hepatic GSH levels were determined. APAP alone caused severe liver injury with peroxynitrite formation and DNA fragmentation, all of which was attenuated by all treatments. However, GSH (-82%) and the amino acids (-79%) were more effective than NAC (-46%) in preventing liver injury. 13C-labeled compounds were administered concomitantly with GSH and amino acids at different time points to measure synthesis rate of GSH and precursors, substrate flux and pathways through the mitochondrial Krebs cycle and tissue ATP levels by NMR (nuclear magnetic resonance) spectroscopy. It was observed that the reduced liver injury with GSH or individual amino acid treatment correlated with an enhanced GSH recovery rate and an increased substrate supply for the mitochondrial Krebs cycle compared to APAP alone. Consequently, hepatic ATP levels were fully restored after treatment with GSH or amino acids compared to a 35% and 56% reduction, respectively, at 1.5 and 6 h after APAP treatment alone. In contrast, NAC had only a partial effect on these parameters, which correlated with the limited protection. Thus, delayed treatment with GSH and NAC protect against AAP overdose by a dual mechanisms, i.e. by enhancing hepatic GSH levels (scavenging of NAPQI and reactive oxygen) and by supporting mitochondrial energy metabolism.

627 COX-2 KO MICE ARE SUSCEPTIBLE TO SPLA2-MEDIATED PROGRESSION OF ACETAMINOPHEN HEPATOTOXICITY.

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Previous studies identified calpain as a mediator of progression of liver injury initiated by CCl4 and acetaminophen (APAP). Here, we report secretory phospholipase A2 (sPLA2) as another mediator of progression of injury initiated by CCl4 in rats. Upon toxicant-initiated necrosis, perinecrotic cells secrete sPLA2 that rids the tissue of necrotic cell debris. Action of sPLA2 releases arachidonic acid, a precursor of protective prostaglandins. Hence, co-presence of sufficient COX-2 with sPLA2 is critical in post-necrotic cleanup and tissue recovery. The aim of our present study was designed to test the hypothesis that the action of sPLA2 spreads hepatocyte injury in the absence of sufficient COX-2 using COX-2 knockout (KO) mice. COX-2 wild type (WT) and KO mice were administered a normally non lethal dose (400 mg/kg) of APAP. The COX-2 KO mice suffered 60% mortality as compared to 100 % survival of the WT mice, suggesting greater APAP toxicity in COX-2 KO mice. Liver injury was significantly higher in the KO mice at later time points as compared to the WT. This difference was not due to increased bioactivation of APAP as indicated by unchanged CYP2E1 protein and covalently bound 14C-APAP in the KO mice. Hepatic sPLA2 activity in the KO mice at 12 and 24 h after APAP administration was significantly higher than in the WT mice. Plasma TNF-α rose in the KO mice throughout the time course vs WT mice, with a corresponding fall in hepatic PGE2. Liver regeneration (3H-Thymidine incorporation) was inhibited in the KO mice vs the WT. These results suggest that there was inhibited PG production in the COX-2 KO mice as compared to the WT mice that further hindered compensatory tissue repair and resolution of inflammation, thus making the liver more susceptible to sPLA2-mediated progression of liver injury. These findings are consistent with the destructive role of sPLA2 in the spread of injury by hydrolysis of plasma membrane phospholipids of perinecrotic hepatocytes unless mitigated by co-presence of sufficient COX-2.

R 628 IMMUNOTOXICITY TESTING: SHOULD ELEVATED ANTIBODY RESPONSES BE INTERPRETED AS AN INDICATOR OF IMMUNOTOXICOLOGICAL HAZARD?

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The current EPA Office of Prevention, Pesticides and Toxic Substances draft Health Effects Test Guidelines (OPPTS 870.7800 Immunotoxicity) were designed to meet testing requirement of the Toxic Substances Control Act and the Federal Insecticide, Fungicide and Rodenticide Act. The guidelines require evaluation of the antibody response to sheep red blood cells (SRBC) to determine whether repeated exposure to a pesticide causes immunotoxicity. The purpose of OPPTS 870.7800 is "to provide information on suppression of the immune system". Although many immunotoxicants suppress the primary antibody response, certain pesticides (e.g., propanil and malathion), drugs (cocaine) and other chemicals of environmental concern (T-2 toxin, cadmium, lead and methyl mercury) have been reported to cause increased responses to T-dependent antigens. It can be argued that increased antibody production in response to immunization should not be interpreted as adverse 'per se' because current vaccination protocols typically rely on chemical adjuvants that stimulate antibody production. However, in some cases, elevated antibody synthesis following xenobiotic exposure has been associated with increased production of autoantibodies or worsening of disease in autoimmune-prone animal models. The current language of the immunotoxicity testing guidelines identifies suppression, rather than modulation, of the antibody response as the outcome of concern, and in essence ignores an increased antibody response to SRBC as a signal of potential immunotoxicity. This Roundtable will address whether considering only suppression of the antibody response may compromise hazard identification and risk assessment.

HH 629 OZONE TOXICOLOGY: HISTORICAL PERSPECTIVES OF THE SCIENCE THAT SHAPED THE REGULATORY STANDARDS.

M. C. Madden. *ORD, NHEERL, HSD, Clinical Research Branch, U.S. EPA, Chapel Hill, NC.*

Ozone (O3) is a ubiquitous air pollutant that has been the focus of intense study for decades. O3 is a criteria pollutant requiring the US EPA to reevaluate new findings on public health periodically to determine if the National Ambient Air Quality

Standard (NAAQS) should be revised. Early studies demonstrated human lung physiological changes in healthy adults. Panel studies with populations exposed to O3 for several hours provided further evidence of ozone-induced physiological changes with ambient exposures. O3-induced changes in human lung cell populations and soluble mediator production in lavage fluids were also documented. In vitro O3 exposures performed with lung cells allowed mechanistic examination of how this pollutant exerted effects. Studies with nonhuman animal models also provided valuable insights into the toxicity. Chief among those types of studies were subchronic and chronic exposure studies, and assessment of whole lung pathobiology. With advances in genetics and molecular biology, the role of genetic polymorphisms in mediating the effects of O3 has been studied, in part to identify possible susceptible individuals. Epidemiological studies continue to examine possible effects of long term ozone exposures, as well as the role of O3 in modifying the effects of other air pollutants, e.g., particulate matter. The reactions of O3 with other airborne components may induce some health effects. O3 has remained a major air pollutant of concern in the U.S. and globally despite regulation and extensive study. For instance, under a recent court ordered decision, the US EPA has until March 12, 2008 to decide to retain or revise the O3 standard. The lessons learned from O3 research have helped optimize current investigations examining the role of other air pollutants in human health effects, have assisted in strategies for performing risk assessment especially with possible sensitive populations, and given guidance in management of health risks. Mort Lippmann, Rich Schlesinger, and Harvey Richmond will present these findings. [This abstract may not reflect EPA policy.]

IS 630 PUTTING YOUR BEST FOOT FORWARD: JOB INTERVIEWING WORKSHOP FOR EARLY-CAREER SCIENTISTS.

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You've finished graduate school and maybe completed one or two stints as a postdoctoral fellow. You may know how to conduct research, write manuscripts and present seminars, but do you know what it takes to land that first job? This interactive workshop is designed to provide novice job seekers with timely and valuable information about how to successfully navigate the hiring process. Listen as hiring managers from government and industry discuss what they look for when reviewing job applications and get the straight facts about what can make or break a job interview. Find out what to say and how to present yourself during the interview so that hiring managers know that YOU are the person for the job. Furthermore, listen to the firsthand experience of an early-career scientist who recently landed an academic position. Watch and learn as experts and audience members participate in skits and mock interviews. Join us for a dynamic, informative and interactive workshop designed to put you in the driver's seat of your career development!

R 631 BREAKING THE LOG-JAM: PUBLIC-PRIVATE PARTNERSHIPS AS A WAY TO DISCOVER AND ADVANCE BIOMARKERS OF DRUG-INDUCED TOXICITY.

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The drug-development process entails an interaction between the industrial (private) sector and public regulatory agencies. Likewise, changing this process through the use of innovative new safety tests also requires an interaction between the public and private sectors. Two unique and parallel efforts are the Innovative Medicines Initiative and the Predictive Safety Testing Consortium. The former, under the auspices of the European Union, brings together academics and companies in a combined effort to discover and qualify new -omics-based markers of toxicity. The latter, under the direction of the non-profit Critical Path Institute, brings together companies to comprehensively qualify new safety biomarkers for both non-clinical and clinical applications. Both efforts directly involve members of the regulatory agencies so that the processes are not just transparent to regulatory agencies, but result in assays that are accepted by the general scientific, industrial and regulatory communities. This Roundtable will provide an overview of these processes with particular attention to the interaction between scientific approaches and regulatory input, and allow discussion of how these partnerships achieve their goals.

R 632 REPRODUCTIVE TOXICITY STUDIES: ONE GENERATION *VERSUS* TWO GENERATIONS.

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As an outcome of an international effort to develop an alternative protocol for hazard and dose-response characterization for agricultural chemicals, a life stage oriented testing paradigm was proposed (Cooper et al., 2006). The screening protocol as described in this manuscript incorporates multiple endpoints that might traditionally be assessed in completely separate studies; it thereby strives to reduce the number of animals used in testing and to refine the testing paradigm through more efficient utilization of animals. An important and controversial aspect of this study design is the use of a single generation of rodents to assess reproductive toxicity. Questions and concerns have been expressed regarding the adequacy of using a one-generation study for the characterization of reproductive hazard and dose response, rather than the traditionally accepted two generations. A previous EPA workshop (Francis and Kimmel, 1988) had concluded that two-generations were optimal and that one would be insufficient. However, a preliminary review of historical evidence available at the time of the Cooper et al. publication suggested that this conclusion may not be universally supported. More rigorous retrospective reviews of reproductive toxicity study outcomes and their use in risk assessment have been conducted since the time of the publication and will be presented in this session. Some of the issues for consideration and discussion include: What has been learned through the retrospective review of reproductive toxicity study data? How might hazard and dose-response characterization be affected by assessment of only one generation? What would trigger the conduct of a second generation, and how confident could one be that this decision would be applied appropriately and consistently? What role might chemical class or mode of action information play in directing the testing paradigm on a chemical-specific basis? How will testing and validation of this approach be conducted?

R 633 THE 2007 PET FOOD RELATED TOXIC NEPHROPATHY IN DOGS AND CATS.

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The focus of this Roundtable will be to provide an up-to-date overview of the pet food related toxic nephropathy that swept across the United States and Canada in 2007. This episode was unique in both the breadth and rapidity of its occurrence as well as to the unique nature of its toxicological manifestations. It exposed the public to a large-scale toxicological investigation in progress, and has had significant impact on assessing safety of imported foods for both animal and human consumption. This Roundtable will review the epidemiology, clinical manifestations, pathology and potential mechanisms of action, as well as the regulatory response and management of the crisis.

IS 634 DETECTION OF BIOLOGICAL FREE RADICALS IN TIME AND SPACE.

M. Waalkes[1], R. Mason[2] and V. Kagan[3]. *[1]LPC, NIEHS, Research Triangle Park, NC, [2]LCC, NCI at NIEHS, Research Triangle Park, NC and [3]University of Pittsburgh, Pittsburgh, PA.*

The accurate and sensitive detection of biological free radicals in a reliable manner is a requirement to define the mechanistic roles of such species in toxicology. Most of the techniques currently available are either not appropriate to detect free radicals in cells and tissues due to sensitivity limitations (electron paramagnetic resonance, EPR) or subject to artifacts that make the validity of the results questionable (flourescent probe based analysis). The recently introduced immuno-spin trapping technique is based on the fact that amino acid derived radicals react with the spin trap 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) to form protein-DMPO nitroxide radical adducts. These adducts have limited stability and decay producing the very stable protein-DMPO-nitrone product. The formation of protein-DMPO-nitrone adducts is based on the selective reaction of free radical addition to the spin trap and is thus not subjected to artifacts frequently encountered with ordinary methods for free radical detection. Rabbit polyclonal anti-DMPO nitrone antiserum has been developed and validated. Immuno-spin trapping is proving to be a potent, sensitive, and accessible method to detect low levels (e.g. nanomolar) of protein-derived radicals produced in vitro and in vivo. Moreover, it has been established that anti-DMPO nitrone antibodies can be utilized for the detection of DNA-derived radicals in the form of DNA-DMPO nitrone adducts, and such

technique has been applied to models of metal carcinogenesis. Most recently, immunofluorescence and immunochemistry have been used to determine the distribution of free radicals in cells and tissues. In summary, the advances the immunospin trapping technique affords in accurate and sensitive determination of biological free radicals is having a major impact on our understanding of the role of such radicals in toxic response and mechanism.

S 635 MOLECULAR AND GENOMIC INSIGHTS INTO THE NRF2-REGULATED OXIDATIVE STRESS RESPONSE: IMPACT ON CARCINOGENESIS.

C. Corton[1] and T. Kensler[2]. [1]*NHEERL, U.S.-EPA, Research Triangle Park, NC and* [2]*Johns Hopkins University, Baltimore, MD.*

The transcription factor Nrf2 plays a significant role in protecting cells from endogenous and exogenous stresses. Mice lacking Nrf2 are more sensitive to the hepatic, pulmonary, ovarian, and neurotoxic consequences of acute exposures to environmental agents and drugs as well as chronic exposures to cigarette smoke and other carcinogens. Under quiescent conditions, Nrf2 interacts with the actin-anchored protein Keap1, leading to low basal expression of Nrf2-regulated genes. However, upon recognition of chemical signals imparted by oxidative and electrophilic molecules, Nrf2 is released from Keap1, escapes proteasomal degradation, translocates to the nucleus, and trans-activates the expression of several dozen cytoprotective genes that enhance cell survival. This symposium will highlight recent exciting findings in the field including different mechanisms by which chemical exposure can activate Nrf2, the role of Nrf2 in modifying chemical carcinogenesis and how cancer cells can highjack this protective system to increase survival.

S 636 FUNCTION AND SIGNAL TRANSDUCTION OF NRF2 FROM A METAL'S PERSPECTIVE.

Q. Ma and X. He. *Toxicology and Molecular Biology Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Morgantown, WV.*

Metals comprise a large group of environmental and occupational toxicants; many are human carcinogens. Although the mechanism by which metals cause cancer and toxicity remains unclear, oxidative damage is known to be a critical component of metal action. In recent years, Nrf2 has emerged as a prooxidant/antioxidant-activated receptor/transcription factor that integrates a variety of oxidative stimuli from both exogenous and endogenous sources and coordinately regulates the antioxidant response element-dependent transcription of a battery of cytoprotective genes, thereby protecting cells from oxidative stress and damage. The function and signal transduction of Nrf2 in response to carcinogenic metals were analyzed in this study, with focus on transcriptional protective mechanisms against metal-induced oxidative stress by Nrf2 and the molecular events of Nrf2 activation by toxic metals. Exposure to carcinogenic metals such as As, Cr, and Cd induces apoptosis and increased production of ROS. Loss of Nrf2 by gene knockout and SiRNA knockdown markedly increases the ROS production and cell death in the presence of the metals. Metals activate Nrf2 by stabilizing the Nrf2 protein via a signaling mechanism distinctively different from that of phenolic antioxidants in that metals disrupt Nrf2/Keap1 association in the nucleus whereas phenolic inducers do not. Both Nrf2 and Keap1 are ubiquitinated in the cytoplasm; upon activation, the two proteins translocate into the nucleus in a complex and are deubiquitinated in the nucleus. Chromatin immunoprecipitation revealed binding of Nrf2 and Maf G/K to the ARE located in the enhancers of Nqo1 and Ho1 for gene transcription. The findings suggest a unique molecular model of metal action in metal sensing and transcriptional gene regulation in mammalian cells.

S 637 REDOX REGULATION OF NRF2: *IN VITRO* SIGNALING CIRCUITRY AND *IN VIVO* CARCINOGENESIS/ANTI-CARCINOGENESIS FUNCTIONS.

T. Kong. *The State University of New Jersey, Piscataway, NJ.* Sponsor: C. Corton.

The bZIP transcription factor Nrf2 plays an important role in antioxidant defense mechanisms against oxidative and electrophilic stresses involved in many disease processes including cancer. Many dietary cancer preventive compounds and exogenous/endogenous oxidants can activate cellular Nrf2 and enhance its nuclear transcriptional activities through redox signaling of a combination of signaling proteins including Keap1, PKC, MAPK, PI3K, GSK, FYN depending on the types of the cells and stimuli. Activation of Nrf2 induces phase II detoxifying/antioxidant enzymes GST, QR, HO-1 and GCS as well as genes involved in ubiquitination, electron transport, transporters, cell growth and apoptosis, cell adhesion, kinase and phosphatases, transcription factors and co-regulators. The overall biological functions would appear to afford cellular protection against oxidative and carcinogenic damages, at least in normal tissues such as the liver and intestine. Consistent with this, Nrf2-/- mice are more prone to carcinogen-induced cancers including skin, colon and other cancers, and Nrf2 is required for the protective effects of many cancer preventive compounds. Additionally, Nrf2-/- mice are more susceptible to dextran sulfate-induced colon inflammation in part due to decreased expression of antioxidant enzymes coupled with enhanced expression of inflammatory cytokines and mediators. The regulation of antioxidant and inflammatory (whether directly or indirectly) signaling by Nrf2 would dictate the carcinogenesis versus the anti-carcinogenesis effects in response to external environmental cues. (Supported by NIH grant R01-CA094828).

S 638 NRF2-MEDIATED SIGNALING: ROLE IN LUNG INFLAMMATION AND TUMORIGENESIS.

S. R. Kleeberger[1], A. K. Bauer[2], X. Wang[1] and D. A. Bell[1]. [1]*NIEHS, Research Triangle Park, NC and* [2]*Michigan State University, East Lansing, MI.* Sponsor: C. Corton.

NRF2-mediated signaling is critical to protection of the lung against oxidative stress-induced injury and inflammation. Chronic lung inflammation is also an important determinant of tumorigenesis. Recent evidence has suggested that NRF2 protects against the pathogenesis of lung injury and inflammation in response to oxidant stimuli, but may contribute to the development of tumors. However, the mechanisms of NRF2 signaling in pulmonary diseases are not completely understood. To begin to address this question, we have developed in silico computational tools for discovering functional single nucleotide polymorphisms (SNPs) in transcription factor binding sites that may impact target gene expression. In this presentation, we discuss the integrated application of computational tools and knockout mouse models to investigate NRF2 effector mechanisms in models of lung injury, inflammation, and tumorigenesis.

S 639 DYSFUNCTIONAL KEAP1-NRF2 INTERACTIONS IN LUNG CANCER AND IMPLICATIONS FOR OTHER CANCERS.

S. Biswal. *Johns Hopkins University, Baltimore, MD.* Sponsor: C. Corton.

Nuclear factor erythroid-2 related factor 2 (NRF2) is a redox-sensitive transcription factor that positively regulates the expression of genes encoding antioxidants, xenobiotic detoxification enzymes, and drug efflux pumps, and confers cytoprotection against oxidative stress and xenobiotics in normal cells. Kelch-like ECH-associated protein 1 (KEAP1) negatively regulates NRF2 activity by targeting it to proteasomal degradation. Increased expression of cellular antioxidants and xenobiotic detoxification enzymes has been implicated in resistance of tumor cells against chemotherapeutic drugs. Systematic analysis of the KEAP1 genomic locus in lung cancer tumors from patient and cell lines revealed deletion, insertion, and missense mutations in functionally important domains of KEAP1 and a very high percentage of loss of heterozygosity at 19p13.2, suggesting that biallelic inactivation of KEAP1 in lung cancer is a common event. Sequencing of KEAP1 in lung cancer cell lines and non-small-cell lung cancer (NSCLC) samples revealed somatic mutations in KEAP1 gene. All the mutations were within highly conserved amino acid residues located in the Kelch or intervening region domain of the KEAP1 protein, suggesting that these mutations would likely abolish KEAP1 repressor activity. Evaluation of loss of heterozygosity at 19p13.2 revealed allelic losses in 61% of the NSCLC cell lines and 41% of the tumor samples. Decreased KEAP1 activity in cancer cells induced greater nuclear accumulation of NRF2, causing enhanced transcriptional induction of antioxidants, xenobiotic metabolism enzymes, and drug efflux pumps. Biallelic inactivation of KEAP1 is a frequent genetic alteration in NSCLC. More recently, we and others have found mutations in KEAP1 gene in prostate and breast cancer. This presentation will focus on presenting evidence that loss of KEAP1 function leading to gain of NRF2-mediated gene expression in cancer helps tumor cells to manipulate the NRF2 pathway for their survival against chemotherapeutic agents and radiotherapy.

S 640 KEAP1-NRF2 SIGNALING AS A TARGET FOR CANCER CHEMOPREVENTION: PRE-CLINICAL AND CLINICAL PERSPECTIVES.

T. W. Kensler. *Environmental Health Sciences, Johns Hopkins University, Baltimore, MD.*

The development of Nrf2 knockout mice provided the first key insights into the toxicological importance of this transcription factor signaling pathway. As examples, Nrf2 knockout mice are more sensitive to the carcinogenicity of benzo[a]pyrene and N-nitrosobutyl-(4-hydroxybutyl)-amine in the forestomach and bladder, respectively. Nrf2-regulated genes govern a broad-based adaptive response to oxidant and electrophilic stresses. Nrf2-mediated transcription of these genes is activated by a number of cellular stresses (endoplasmic reticulum stress, ox-

idative stress, and shear stress), signaling molecules (nitric oxide, growth factors) and oxidized lipids (15-deoxy-delta-12,14-prostaglandin J2, oxidized low density lipoproteins) leading to increased stability and nuclear accumulation of Nrf2. In some instances, cysteines on Keap1, a cytoplasmic tether for Nrf2, serve as sensors leading to pathway activation. Of great relevance to disease prevention, this pathway also can be induced by an expanding array of small molecule drugs and natural products with little toxicity including dithiolethiones (e.g., oltipraz), isothiocyanates (e.g., sulforaphane) and triterpenoids (e.g., CDDO-Im). Monitoring for induction of Nrf2-regulated genes led to the original identification of oltipraz as a potential chemopreventive agent and, more recently, the recognition that some triterpenoids are exceptionally potent inhibitors of carcinogenesis. In addition, the anticarcinogenic efficacies of these agents are lost in Nrf2 knockout mice, highlighting the central importance of this protective pathway. Several of these inducers are currently being evaluated for pharmacodynamic action in clinical trials for cancer prevention through analysis of altered disposition of environmental carcinogens in exposed individuals. Supported by ES06052, CA39416, CA94076 and Breast SPORE CA88843.

S 641 NEW CONCEPTS IN THE ETIOLOGY OF BREAST CANCER: FROM GENES TO ENVIRONMENT AND BACK AGAIN.

D. L. Eaton. *Env. Occup. Health Sciences., University of Washington, Seattle, WA.*

This 'all-star' cast of breast cancer researchers will present new research that provides important new insights into the etiology of breast cancer. One out of eight woman living in the US today will get breast cancer sometime in her lifetime. Although mortality from breast cancer has declined somewhat over the past 10 years due to earlier diagnosis and better treatment, some studies indicate that the incidence increased in this same period. The discovery of the BRCA1 gene by Mary-Claire King and others over 15 years ago has led to the identification of additional, but generally less penetrant, breast cancer genes, such as CHEK2, ATM, NBS1, RAD50, BRIP1, and PALB2. However, most breast cancer is non-familial and the etiology is multifactorial. Although lifestyle factors that influence hormonal regulation are undoubtedly important, environmental factors such as organochlorine pesticides, phyto-estrogens, and diurnal rhythm have also been hypothesized to contribute to the etiology of breast cancer. This symposium will present new data on 'established' risks factors, as well as explore new concepts and approaches to unraveling the genetic and environmental causes of this disease. The symposium will be geared to a general audience, but will also provide new information on emerging environmental risk factors for this important disease.

S 642 NEW INSIGHTS INTO HORMONAL, HISTOLOGICAL, AND MOLECULAR ASPECTS OF BREAST CANCER ETIOLOGY.

C. I. Li. *Epidemiology, Fred Hutchinson Cancer Research Center, Seattle, WA.* Sponsor: D. Eaton.

Despite often being treated as a single disease in etiologic studies, breast cancer is a heterogeneous disease that can be categorized in several ways. Recent epidemiologic studies suggest that established breast cancer risk factors differ considerably in their associations with breast cancer subtypes defined according to hormone receptor status and histological type. Molecular profiling studies have also identified clinically relevant breast cancer subtypes that are associated with substantially different risks of mortality. Our work has focused on identifying novel risk factors for various subtypes of breast cancer based on hormone receptor expression, histology, and molecular profile. For example, we have found that use of menopausal hormones is more strongly related to risk of invasive lobular carcinoma than it is to other histological subtypes of breast cancer, and that obesity is more strongly related to risk of basal-like, or so-called triple negative, tumors which is the molecular subtype of breast cancer that has the poorest survival. Further studies aimed at advancing our understanding of the etiologies of different types of breast cancer are needed.

S 643 CIRCADIAN DISRUPTION AND THE RISK OF BREAST CANCER.

S. Davis[1, 2]. [1]*Epidemiology, University of Washington, Seattle, WA and* [2]*Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA.* Sponsor: D. Eaton.

There is interest in the possibility that disruption of normal circadian rhythm may increase the risk of cancer. Persons who work nightshifts may exhibit altered nighttime melatonin levels and reproductive hormone profiles that could increase the risk of breast cancer. Epidemiologic studies suggest that women who work at night, and who experience sleep deprivation, circadian disruption, and exposure to light-at-night are at an increased risk of breast cancer. Several studies in Seattle have investigated factors that can disrupt circadian rhythm and alter normal nocturnal production of melatonin and reproductive hormones of relevance to breast cancer etiology. These studies have found: 1) an increased risk of breast cancer associated with exposure to light-at-night and night shift work; and 2) decreased nocturnal urinary levels of 6-sulphatoxymelatonin associated with exposure to 60-Hz magnetic fields, and other factors including hours of daylight, season, alcohol consumption and body mass index. A crossover study designed to investigate whether exposure to a 60-Hz magnetic field under controlled conditions in the home sleeping environment is associated with a decrease in nocturnal urinary melatonin, and an increase in the urinary levels of luteinizing hormone, follicle stimulating hormone, and estradiol in a sample of healthy women of reproductive age has recently been completed. Presently underway is a study to determine whether working at night is associated with decreased levels of urinary melatonin and increased levels of the reproductive hormones listed above in a sample of healthy women of reproductive age, and to elucidate characteristics of sleep among night shift workers related to the hormone patterns identified. A new study of similar design in a sample of healthy men will also investigate whether polymorphisms of the genes thought to regulate the human circadian clock are associated with the ability to adapt to night shift work. Results from these studies will enhance our understanding of the role of circadian disruption in the etiology of cancer.

S 644 GENOMIC ANALYSIS OF INHERITED BREAST CANCER.

M. King. *Genome Sciences, University of Washington, Seattle, WA.* Sponsor: D. Eaton.

Inherited breast cancer is associated with germline mutations in at least ten different genes in pathways critical to genomic integrity. Mutations in BRCA1 and BRCA2 confer very high risks of breast and ovarian cancer. Inherited mutations in p53 and PTEN, which are very rare, lead to very high breast cancer risks associated with the Li-Fraumeni and Cowden syndromes, respectively. Inherited mutations in CHEK2, ATM, NBS1, RAD50, BRIP1, and PALB2 are associated with 2-fold to 4-fold increases in breast cancer risks. In addition, biallelic mutations in BRCA2, BRIP1, and PALB2 can cause Fanconi anemia. The convergence of these genes in a shared role reveals underlying biology of these illnesses and suggests still other breast cancer genes.

S 645 THE ONCOGENIC MICRORNA-27A TARGETS GENES THAT REGULATE SPECIFICITY PROTEIN (SP) TRANSCRIPTION FACTORS AND ESTROGEN RECEPTOR α IN BREAST CANCER CELLS.

S. Safe[1, 2], S. U. Mertens-Talcott[1], S. Chintharlapalli[2] and X. Li[1]. [1]*Veterinary Physiology & Pharmacology, Texas A&M University, College Station, TX and* [2]*Institute of Biosciences & Technology, Texas A&M Health Science Center, Houston, TX.*

There is evidence that specificity proteins (Sps), such as Sp1, Sp3 and Sp4, are overexpressed in tumors and contribute to the proliferative and angiogenic phenotype associated with cancer cells and are important for hormonal activation of genes through the estrogen receptor α (ERα)/Sp complex. Sp1, Sp3 and Sp4 are expressed in a panel of estrogen receptor (ER)-positive and -negative breast cancer cell lines, and we hypothesized that regulation of their expression may be due to microRNA-27a (miR-27a) which is also expressed in these cell lines and has been reported to regulate the zinc finger ZBTB10 gene, a putative Sp-repressor. Transfection of ER-negative MDA-MB-231 breast cancer cells with antisense miR-27a (as-miR-27a) resulted in increased expression of ZBTB10 mRNA and decreased expression of Sp1, Sp3 and Sp4 at the mRNA and protein levels and also decreased activity in cells transfected with constructs containing Sp1 and Sp3 promoter inserts. In addition, these responses were accompanied by decreased expression of Sp-dependent survival and angiogenic genes including survivin, vascular endothelial growth factor (VEGF) and VEGF receptor 1 (VEGFR1). Moreover, similar results were observed in MDA-MB-231 cells transfected with ZBTB10 expression plasmid. In addition, we observed that miR-27a also plays an important role in ER-positive MCF-7 breast cancer cells, and transfection with as-miR-27a not only decreases Sp proteins but also expression of ERα and hormone-dependent genes. These results demonstrate the oncogenic activity of miR-27a in breast cancer, and similar effects have been observed in many other solid tumors. In addition, results of ongoing studies have identified drugs that target miR-27a.

S 646 NETWORKS OF PROTEIN-PROTEIN INTERACTIONS NECESSARY FOR UBIQUITINATION OF ESTROGEN RECEPTOR ALPHA BY BRCA1/BARD1.

C. M. Eakin[1], M. J. MacCoss[2], D. E. Christensen[1] and R. E. Klevit[1]. [1]*Biochemistry, University of Washington, Seattle, WA and* [2]*Genome Sciences, University of Washington, Seattle, WA.* Sponsor: D. Eakin.

Cellular processes ranging from protein degradation to viral vesicle budding are regulated by attachment of the small protein ubiquitin to protein substrates. Ubiquitin attachment to a protein is carried out via several steps and a well-known trio of enzymes called ubiquitin activating (E1), ubiquitin conjugating (E2), and ubiquitin

ligase (E3). We investigated the protein networks utilized by the E3 ligase, BRCA1/BARD1, that allow for the specific ubiquitination of a substrate. The breast cancer suppressor protein, BRCA1, is expressed in a wide range of tissues, but is associated with the development of tissue-specific cancers in the breast and ovaries. Recent studies have suggested this tissue-specificity may be linked to inhibition of estrogen receptor alpha (ERα) transcriptional activation by BRCA1. We show ERα is a substrate for the BRCA1/BARD1 ubiquitin ligase, suggesting a possible mechanism for regulation of ERα activity by BRCA1. The regions of BRCA1/BARD1 and ERα necessary for ubiquitination are determined. In addition, the specificity of both E2 and substrate interactions with BRCA1 for ERα ubiquitination are evaluated. As ERα is the only known BRCA1/BARD1 substrate that shares an expression profile with BRCA1-associated tumors, the identification of ERα as a BRCA1/BARD1 substrate reveals a potential link between the loss of BRCA1/BARD1 ligase activity and tissue-specific carcinoma.

W 647 DRUG-RELATED TORSADES DES POINTES: ADVANCEMENTS IN PRECLINICAL MODELING OF POTENTIAL CARDIAC TOXICITY.

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Drug-induced delay in cardiac ventricular repolarization (QT/QTc prolongation) serves as a sensitive surrogate of the potential life-threatening cardiac arrhythmia, torsades des pointes (TdP), but importantly lacks the specificity to know if, when & under what circumstances TdP may be elicited in the clinical population. Identification of better preclinical models of drug-related TdP is a significant challenge for the scientific community, and was thoroughly debated at an ILSI/HESI workshop convened in November 2005 at which recommendations for further study were proposed (www.hesiglobal.org/ Events/TdPWorkshop.htm). Advancements in three key areas of investigation will be judged in terms of progress in each of these areas of model development. They include: cellular and molecular biology of drug-induced TdP, attempts to directly link drug exposure to TdP; understanding the dynamics of periodicity (beat to beat variability in the electrocardiogram) from preclinical and clinical investigations; and in vitro and in vivo modeling of drug-induced TdP. The timing of the 2008 SOT workshop is important as 2009/2010 was selected for a follow up two day ILSI/HESI meeting at which those areas of scientific pursuit holding the greatest promise of providing better predictors of TdP arrhythmia will be considered and possibly prioritized. The 2008 workshop will be an important interim assessment of advancements in each of the three areas of study since the 2005 program and also serve to advocate further investigation by toxicology scientists in preparation for the important debate in 2009/2010. Drug-induced TdP remains a serious public health issue in rapidly bringing safe new pharmaceutical agents to the marketplace for diseases for which there are no acceptable alternative therapies. As a result, accurate identification of those drugs posing a risk of cardiac toxicity using the best preclinical models is of paramount importance. This workshop is being co-sponsored by Society of Toxicology and Safety Pharmacology Society.

W 648 CELLULAR AND MOLECULAR BIOLOGY OF DRUG-INDUCED TDP: THE ROLE OF POTASSIUM CHANNELS, OTHER PROTEINS, AND DOWNSTREAM EVENTS.

D. Roden. *Oates Institute for Experimental Therapeutics, Vanderbilt University School of Medicine, Nashville, TN.* Sponsor: A. Bass.

A balance between inward and outward currents through multiple ion channels in individual ventricular myocytes determines action potential duration (APD). Decreased net outward current increases APD and results in QT prolongation. This can be an acquired lesion (usually due to drugs) or congenital, as in the congenital long QT syndromes. Drugs almost always prolong the QT interval by blocking one specific outward current in heart, IKr. Individual cells vary in the extent to which a drug challenge prolongs action potential, likely reflecting variability in the magnitude of multiple currents among cells. When action potentials become especially prolonged, inward currents (whose identities are incompletely defined) can increase, resulting in positive feedback, further action potential prolongation, early afterdepolarizations, and spontaneous cellular depolarizations. When this occurs in only some cells, and action potentials are short in other cells, such abnormal depolarizations can propagate, creating the unstable reentry that is thought to underlie torsades de pointes. We have hypothesized that redundancies in cardiac repolarization protect normal hearts against long QT-related arrhythmias and that loss of such redundancies ("reduced repolarization reserve") thus contributes to genetic or acquired individual variability in susceptibility to long QT-related arrhythmias. This framework identifies functional variations (genetic or acquired) in not only the IKr channel complex but also other ion channel proteins, and their interacters, as candidate modulators of risk for these arrhythmias. Moreover, the concept emphasizes that a reduction in IKr is simply the first link in a chain of molecular events that can culminate in torsades de pointes.

W 649 CELLULAR AND MOLECULAR BIOLOGY OF DRUG-INDUCED TORSADE DE POINTES (TDP) IN THE LONG QT SYNDROME: ROLE OF CALCIUM CHANNEL MODULATION IN TRIGGERING TDP.

G. Salama. *Cell Biology and Physiology, University of Pittsburgh, Pittsburgh, PA.* Sponsor: A. Bass.

Inherited and drug induced forms of the long QT (LQT) syndrome are characterized by a great deal of variability in arrhythmia phenotype and sudden cardiac death (SCD). To better understand the co-factors that influence the arrhythmia phenotype in LQT2, the mechanisms underlying sex differences in TdP vulnerability were investigated in rabbit hearts since as in humans, adult females have a higher risk to TdP than males and the arrhythmia phenotype is reversed in pre-puberty before the surge of sex hormones. In both pre-pubertal and adult rabbits, females have longer APDs due to lower levels of IKr yet exhibit opposite arrhythmia phenotypes implicating mechanisms other than QT prolongation.
Early afterdepolarizations (EADs) that fire during the plateau phase of the action potential (AP) are known to trigger TdP and simultaneous maps of voltage and intracellular Ca2+ revealed that spontaneous Ca2+ release from the sarcoplasmic reticulum precede and trigger EADs most likely by activating the Na/Ca exchange current, INCX which in turn triggers the L-type Ca2+ current, ICa,L. To elucidate the role of Ca2+ handling as a co-factor in predicting the arrhythmia phenotype, pharmacological inhibition of INCX, Western and Northern blot analysis of NCX and Ca1.2α, and whole cell current density measurements of INCX and ICa,L under voltage clamp techniques were compared in male versus female adult and pre-pubertal hearts. The analysis revealed that higher ICa,L and INCX in adult females than males and the reversal of these currents in pre-pubertal hearts account for these sex differences. Moreover, single cell measurements and simulations of APs using a modified Luo-Rudy model revealed that the higher ICa,L levels (30%) in adult female myocytes at the base of the heart account for the greater propensity to EADs compared to male myocytes. In conclusion, QT prolongation does not precipitate TdP unless accompanied with intracellular Ca2+ overload and spontaneous SR Ca2+ release which can be attributed to differences in ICa,L and INCX.

W 650 DYNAMICS OF PERIODICITY: PRECLINICAL AND CLINICAL EVIDENCE OF PROARRHYTHMIA POTENTIAL.

A. A. Fossa. *Translational and Molecular Medicine, Pfizer Global Research and Development, Groton, CT.* Sponsor: A. Bass.

The QT/QTc interval prolongation is known to be an unreliable measure of arrhythmia liability. Restitution (relationship of action potential duration to the diastolic interval) measured directly on the heart is a predictor of arrhythmia liability. Studies from our laboratory in conscious dogs indicated that ECG restitution or the beat-to-beat QT and preceding TQ intervals may also provide similar information as the diastolic interval (TQ interval) shortens toward the effective refractory period. To examine the translational changes in humans in varying autonomic states, sotalol was examined over 24 hours. The QT, RR and TQ intervals were examined from sequential cycles in normal male and female volunteers. Additionally, comparisons were made to those observed in a time-matched dataset prior to torsade de pointes from a heart diseased patient that received a single dose of sotalol. In normal subjects, sotalol increased QT, RR and TQ intervals 71, 101 and 125 ms after 160mg and 194, 235 and 135 ms after 320 mg at Cmax, respectively. At baseline, the percentage of beats with a QT/TQ ratio >1 is approximately 20% and was reduced 25% over the entire 22.5 hours after sotalol. The lower TQ interval boundary (5th quantile) was increased 22-30%. Sotalol reduced the magnitude of the maximum QT/TQ ratio by 15-20% during all periods. Since the TQ interval is affected by both the preceeding QT and RR intervals, it would appear the beta-adrenergic blocking activity of sotalol may offset the QT-prolonging effects in normal subjects as opposed to patients with heart disease that are known to have down-regulated beta receptor function. In the time-matched dataset from a heart diseased patient after a single dose of sotalol, 99% of the beats prior to torsade de pointes had a QT/TQ ratio > 1 and the median TQ interval was below the lower 98% confidence bounds of normals before or after sotalol. These data suggest why all QT prolongations should be not considered the same. ECG restitution may allow differentiation/quantification of arrhythmia risk when compared to QT/QTc prolongation alone.

W 651 ADVANCES IN NON-CLINICAL MODELS FOR DETECTING DRUG-INDUCED TORSADES DE POINTES (TdP).

J. Valentin[1], C. Lawrence[1], C. E. Pollard[1], G. Duker[2] and T. G. Hammond[1]. [1]*Safety Assessment UK, AstraZeneca R&D, Alderley Park, Macclesfield, United Kingdom and* [2]*Integrative Pharmacology, AstraZeneca R&D, Molndal, Sweden.*

While it is true that most cases of drug-induced TdP have been associated with hERG channel block, the reverse, that all drugs that block hERG cause TdP, is not true. An equally false assumption is that an increase in the QT interval duration will necessarily lead to TdP. While there is evidence that data from the standard ICH S7B assays is predictive for the risk of QT interval prolongation in man, these assays do not focus on predicting TdP. Published evidence suggest that there may be better predictors of drug-induced TdP in man. Electrophysiological markers considered to be associated with drug-induced TdP other than duration of the action potential and of the QT interval include but are not limited to: triangulation; reverse use-dependence; temporal, spatial and transmural dispersion of ventricular repolarisation; the difference in duration between the peak and the end of the T wave; and the incidence of overt signs of proarrhythmias such as early-after-depolarizations and ectopic beats. Both *in vitro* and *in vivo* proarrhythmia models will be reviewed along with their particular merits and shortcomings. The variables used by each model to predict the torsadogenic propensity of a drug has been reported to be concordant to some extent with the clinical outcome, although data should be interpreted cautiously since not all models have been independently and thoroughly assessed. An enhanced understanding and validation of the key potential proarrhythmic surrogate markers may provide a rational basis for drug progression into clinical development particularly in areas of unmet medical need.

W 652 THE ALLERGIC MARCH: THE ROLE OF CHEMICALS IN THE INCREASING PREVALENCE OF ALLERGY AND ASTHMA.

I. Kimber[1] and I. Gilmour[2]. [1]*Central Toxicology Laboratory, Syngenta, Cheshire, United Kingdom and* [2]*Experimental Toxicology, EPA, Research Triangle Park, NC.*

It is now well established that during the last four decades there has been in industrialized western societies a substantial increase in the prevalence of asthma and atopic allergic disesae. These changes are acknowledged to have been too rapid to be accounted for by alterations in the gene pool, and for this reason there has been considerable interest in the roles played by environmental influences and acquired host factors on susceptibility to allergy and asthma. In this context there has been speculation that increased exposure to certain specific chemicals and/or chemical pollution more generally, may impact on the development of allergy and asthma. We will here explore whether and to what extent chemical exposure is implicated in susceptibility to allergic disease, and in addition what other environmental factors may be influential

W 653 INTRODUCTION: THE CHANGING FACE OF ALLERGY AND ASTHMA.

J. Vanoirbeek. *Department of Public Health, Research Unit for Lung Toxicology, Katholieke University Leuven, Leuven, Belgium.* Sponsor: P. Hoet.

The purpose of this first presentation will be to provide a background to, and basis for, the remainder of the symposium. Terms will be defined and the clinical pictures of allergy and asthma described. The natural history of these diseases will be considered and the changes that have been witnessed in their prevalence during the last 40 years will be discussed – using available epidemiologic data from various origin, including the ISAAC studies. The point will also be made that changes in the prevalence of atopic allergy and asthma have been too rapid to be accounted for on the basis of changes to the gene pool, and for this reason the importance of other factors, including environmental and lifestyle factors, have been attracted considerable interest.

W 654 THE INFLUENCE OF PARTICULATE AIR POLLUTANTS ON ALLERGIC SENSITIZATION IN ANIMAL MODELS.

M. Gilmour. *NHEERL, U.S. EPA, Research Triangle Park, NC.*

Air pollution has long been associated with detrimental health risks in susceptible populations including asthmatics. Experimental evidence in rodents indicates that inhaled or instilled air pollutants such as diesel exhaust particles (DEPs), residual oil fly ash or its constitutive metals, can cause lung injury, inflammation, and potentiate allergic airway responses. These effects which have also been demonstrated with particulates sampled from ambient air are dependent on the underlying chemistry. Genomic analysis of mouse lungs following instillation or inhalation of various particles show broad differences in pathways associated with immune signaling, cell metabolism and oxidative stress. Studies with human airway cell cultures have shown similar alterations in cell signaling and may provide an in vitro high throughput screening framework for predicting allergic adjuvant effects. This presentation will describe a range of environmental factors in urban air that have been implicated as having had a potential impact on susceptibility to the development of atopic allergy and asthma. Included among those considered will be diesel exhaust particles, and ambient air samples. The relative importance of such factors, and the mechanistic basis for their action, will be discussed. (This abstract does not reflect EPA policy)

W 655 THE "HYGIENE HYPOTHESIS" AND BEYOND.

I. Kimber. *Central Toxicology Laboratory, Syngenta, Cheshire, United Kingdom.*

The hygiene hypothesis has its origins in observations made nearly 20 years ago that infections during childhood, acquired for instance by unhygienic contact with older siblings, appear to confer protection against pollen allergy and other forms of atopic disease. In its simplest form the hygiene hypothesis suggests that the increasing prevalence of atopic allergic disease and asthma witnessed during recent decades in westernized countries is attributable largely to reduced exposure to pathogenic microorganisms. The argument is that in the absence of appropriate stimuli, provided normally by bacteria and viral pathogens, the immune system fails to adopt a Th1-type orientation, favoring instead selective Th2 responses resulting in a predisposition to allergic sensitization. Support for the hygiene hypothesis derives from epidemiological studies, including those that have compared discrete geographical locations. These have revealed an association between higher living standards and an increased prevalence of allergic disorders. However, compelling as this hypothesis is, it is likely that factors other than prevailing hygiene conditions, including for instance diet, also impact on susceptibiltiy to atopic allergy and asthma and these will be described also

W 656 PARTICULATES, ALLERGY AND ASTHMA.

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Particles important in relation to allergy and asthma include diesel exhaust particles, wood combustion particles, and mineral particles. Two central but not necessarily related functional aspects of particles are the adjuvant (enhancing) effect on allergic immune responses, and the triggering and enhancing effect on asthmatic responses. Both chemical composition and physical size are particle properties of importance. Focus of the presentation will be on the adjuvant effect and the importance of particle size. Diesel exhaust particles (DEP) have been extensively studied during the last twenty years, and are often used as reference particles in experimental studies. Important features of DEP are the coat of organic substances and metals that cover the carbon core, and the small size and large surface-to-mass and number-to-mass ratios of the particles. Wood smoke particles, in spite of being an abundant type of combustion particles in many regions, have till now been far less studied than DEP both epidemiologically and experimentally. Wood is a renewable, CO_2-neutral source of energy and may therefore become an even more important source of energy in the future. New experimental data will be presented, indicating that wood smoke particles are different from diesel exhaust particles and comparable to road dust particles with regard to adjuvant activity on the specific IgE response to allergen. Mineral particles (crustal material) mainly belong to the coarse fraction of particulate air pollution, and tend to be regarded as less of a problem in relation to health effects than combustion particles. New data from our laboratory indicate that mineral particles dilute out the adjuvant effect of combustion particles in mixed road dust. Finally, interactions between particulate matter and ozone and NOx will be discussed. Experimental results suggest that combined exposure to NO_2 and DEP does not increase the IgE response to allergen more than DEP alone.

W 657 PHTHALATES AND ATOPIC ALLERGY : IS THERE AN EFFECT?

R. J. Dearman. *University of Manchester, Manchester, United Kingdom.*

During recent decades the prevalence of IgE-mediated (atopic) allergic diseases (including respiratory allergy and asthma) in Western Europe and the US has been increasing dramatically. It has been suggested that one possible cause of this increase is the presence in the environment of chemicals that may act as adjuvants, enhancing immune and allergic responses. One group of organic chemicals that has been implicated is the phthalates, used frequently in the manufacture of epoxy resins, plasticizers and adhesives and for which global production has increased during the

last 50 years to approximately 3.5 million metric tons annually. There is some evidence available from epidemiological studies to suggest that increased total levels of phthalates in household dust are found in homes of subjects with asthma and/or allergic rhinitis, although it was not possible to demonstrate a causal relationship. In some experimental (murine) models, particularly those in which phthalate is administered by nonphysiological routes of exposure (subcutaneously, or by intraperitoneal injection), adjuvant effects have been shown on antibody responses or cytokine expression provoked by exposure to a reference allergen (ovalbumin), or to a reference immunogen (keyhole limpit hemocyanin). Other investigators have failed to observe adjuvant effects of phthalates when administered by the topical route of exposure under conditions where marked systemic effects were detected on liver weight. Taken together it seems unlikely that exposure to phthalates is having a substantial impact on the increased incidence of asthma and atopic allergy.

W 658 TO DYE OR NOT TO DYE: SAFETY OF OXIDATIVE HAIR DYES.

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During the past decades, the safety of oxidative hair dyes has been repeatedly questioned. Oxidative hair dyes contain aromatic amines, a chemical family that includes known human carcinogens. Therefore, the association of hair dye exposure and neoplastic diseases in consumers and professionals handling hair dyes has been the subject of numerous epidemiological and toxicological investigations. During the past years several investigators have undertaken major research efforts to investigate the safety of oxidative hair dyes in depth, in particular the systemic exposure of consumers, the metabolism of hair dyes in different human NAT2 genotypes, hair dye metabolism in animal models after dermal application, human skin and hepatocytes, including their capacity to form electrophilic and potentially carcinogenic metabolites. In addition, several recent epidemiologic investigations have implicated oxidative hair dyes, especially the permanent type, in the development of bladder cancer, leukaemia and non-Hodgkin's lymphoma. The objectives of the session are (1) Present and discuss the recent findings from human studies and animal studies; (2) Use of animal data in safety assessment of oxidative hair dyes for human use; (3) Role of genetic polymorphism and hair dye toxicity; and (4) regulatory stipulations with respect to health and safety.

W 659 EPIDEMIOLOGY STUDIES ON HAIR DYES AND CANCER.

K. J. Helzlsouer[1, 2], D. E. Rollison[3] and S. M. Pinney[4]. [1]*Prevention and Research Center, Mercy Medical Center, Baltimore, MD,* [2]*Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD,* [3]*H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL and* [4]*University of Cincinnati College of Medicine, Cincinnati, OH.* Sponsor: M. Soni.

Hair dye products are commonly used world-wide. Permanent hair dyes, the type most commonly used, are formed by an oxidative process involving arylamines, raising investigations concerning long term effects on cancer outcomes. A 1993 International Agency for Cancer Research (IARC) review concluded that evidence was inadequate to evaluate the carcinogenicity of personal hair dye use. Since that review, over thirty articles have been published that examine the association between hair dye use and cancer. These articles were reviewed, and the quality of the exposure assessment in each study was evaluated and rated from 1+ (lowest quality: assessed ever use of hair dyes) to 4+ [highest quality: assessed dye type (permanent/non-permanent), dye color/shade, frequency and duration of use]. Personal hair dye use was associated with increased risks of non-Hodgkin's lymphoma, multiple myeloma, acute leukemia and bladder cancer in at least one well-designed study with high quality exposure assessment (rated 3+ or 4+), although these associations were not consistently observed across studies. Results suggest that subsets of the population may be genetically susceptible to hair dye exposures, but these findings are based on small subgroup analyses and are not consistently observed. Future studies with large sample sizes should include high quality exposure assessment in their design and investigate genetic variation in metabolizing pathways.

W 660 HUMAN EXPOSURE TO [14C]-PARA-PHENYLENEDIAMINE-CONTAINING OXIDATIVE HAIR DYES.

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Hairdressers (N=18) colored training heads bearing natural human hair (length 30 cm) for 6 days, 6 hours / working day with an oxidative hair dye containing 2% [14C]-para-phenylenediamine (PPD). Work phases monitored were A) dye preparation / hair dyeing, B) rinsing / shampooing / conditioning and C) cutting / drying / styling. Ambient air and personal monitoring samples, nasal and hand rinses were collected during all study phases. Urine (0-48 hrs)and blood samples (blank, 4, 8 or 24 hrs) were collected from all study subjects. All samples, study materials, tools and washing liquids were analysed and a [14C]-mass balance was performed. Equipment, gloves and coveralls contained 0.41 ± 0.16%, dye mixing bowls 2.88 ± 0.54%, hair wash 45.47 ± 2.95%, hair + scalp 53.46 ± 4.06% of the applied radioactivity, respectively. Plasma levels were below the limit of quantification (10 ng PPDeq/mL). Total urinary 0-48h excretion of [14C] levels ranged from a total of <3µg to 18 µg PPDeq and was similar in subjects of different phases of hair dyeing. Air levels at or slightly above the limit of quantification were found in a few personal air monitoring samples during the phases of hair dyeing and hair cutting only. Other air samples or nasal rinses contained no measurable radioactivity. Hand residues ranged from 0.006 to 0.15 µg PPD equivalents / cm2, and were found mainly after the cutting/drying phase. The mean mass balance of [14C] across the 6 study days was 102.50 ± 2.20%. The total systemic exposure of hairdressers to oxidative hair dyes during the hair dyeing process was estimated to be <0.33 µg PPDeq/kg body weight / working day. Our results suggest that current safety precautions offer sufficient protection against local and systemic exposure. Overall, our data and the results of previous and ongoing consumer exposure studies suggest that oxidative hair dyes do not pose a risk to human health.

W 661 METABOLISM OF OXIDATIVE HAIR DYES - A KEY CONSIDERATION FOR SAFETY ASSESSMENT.

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The potential carcinogenicity of oxidative hair dyes has been of long-standing interest because these products contain aromatic amines as key ingredients. Certain aromatic amines (4-aminobiphenyl, 2-naphthylamine, benzidine) are known human bladder carcinogens. These known carcinogens require metabolism to form the proximate carcinogen via CYP-mediated N-hydroxylamine formation. In addition, N-acetylation by NAT1 and NAT2 is a well-established pathway for metabolism of many aromatic amines. Therefore, the metabolism of aromatic amines used in oxidative hair dyes is important in the assessment of potential carcinogenicity of these compounds. Recent metabolism studies with hair dye aromatic amines include in vitro studies of hepatic and skin metabolism, in vivo studies via the oral or dermal route, and a clinical study involving hair dye use. The results show that hair dye aromatic amines are substrates for NAT1 and are N-acetylated in the skin. Both in vitro and in vivo studies indicate that N-acetylation is also a predominant hepatic metabolism pathway. Experimental data support the conclusion that N-acetylation of hair dyes represents a detoxification pathway because, unlike the parent aromatic amine, in vitro genotoxicity was not observed with the N-acetylated metabolites. There is also no evidence for hepatic N-hydroxylation typical of the carcinogenic aromatic amines. In vitro and in vivo studies indicate that hepatic phase II conjugation reactions (sulfation, glucuronidation) also represent major pathways for metabolism of some hair dyes, especially after oral administration. NAT2 genotype does not influence the urinary metabolite profile of p-phenylenediamine in human subjects after hair dye use, which is inconsistent with the inference that hair dye use might be associated with an increased bladder cancer risk in NAT2 slow acetylators.

W 662 CARCINOGENICITY HAZARD ASSESSMENT OF HAIR DYES: IS THERE A BIOLOGICALLY PLAUSABLE CANCER RISK?

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Some hair dye ingredients, e.g., 4-amino-2-nitrophenol, have been reported to be carcinogenic when administered to rodents by the oral, gavage, route of administration under standard bioassay conditions, e.g., use of a maximum tolerated dose and lifetime exposure. However, other hair dye ingredients, e.g., 2,5-diaminotoluene, test negative under these conditions. Furthermore, bioassays of hair dyes containing the ingredients found to be carcinogenic by the oral route have yielded uniformly negative results for both carcinogenicity and systemic toxicity when the material was applied topically. These data come from seven publications which report results obtained from three species. Additionally, those hair dye ingredients that were deemed to be rodent carcinogens following oral dosing exhibit relatively low potencies as evidenced by their high TD50 values, the daily dose rate in mg/kg/day to in-

duce tumors in half of the test animals that would have remained tumor free at zero dose (Gold et al., Carcinogenic Potency Project, http://potency.berkeley.edu). Risk assessments for two of the ingredients that were reported to be positive in the rodent bioassay (2,4-diaminoanisole and 4-amino-2-nitrophenol) were performed based on human percutaneous penetration data, using a highly conservative linear, no threshold, extrapolation. These evaluations indicated the cancer risk to be inconsequential. Additionally, it is important to note that the rodent bioassay involves dosing for 5 days/week for a lifetime. This should be contrasted with human use of oxidative hair dyes that are typically applied once/month for a less than lifetime duration. The US Environmental Protection Agency (EPA) Guidelines for Cancer Risk Assessment, 2005, highlight a weight of evidence narrative which emphasizes the importance of considering conditions under which a compound might be carcinogenic (e.g., dose, route and duration of exposure). In this context, the weight of evidence, based on rodent carcinogenicity data, suggests that hair dyes do not present a significant cancer risk for humans.

W 663 CURRENT SAFETY CONCERNS ABOUT OXIDATIVE HAIR DYES: EUROPEAN PERSPECTIVE.

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Based on a recommendation of the former Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) the EU commission took steps to control the use of hair dye chemicals in Europe. An assessment strategy was published including requirements for a safety dossier based on the SCCP's (Scientific Committee on Consumer Products) Notes of Guidance focussed on chemistry, tests for dyes/dye-precursors alone and/or in combination, data on genotoxicity and carcinogenicity. Hair dyes for which no safety files have been submitted were banned (22). 117 dossiers were submitted and 46 were evaluated. Key elements of evaluation are chemistry, genotoxicity, subchronic toxicity, percutaneous absorption and sensitisation. Methodology of genotoxicity, sensitisation and percutaneous absorption were improved but genotoxicity testing of hair dye mixtures remains a challenge. In sum, a) chemistry of hair dyeing is now better understood, b) there is little if any safety concern regarding genotoxicity of the hair dye precursors in use but the reaction mixtures were yet not assessed and c) skin sensitising potential of hair dyes is of concern. A future assessment without animal data is considered unsuitable to ensure human safety.

IS 664 PROFESSIONAL CAREER DEVELOPMENT AS TOXICOLOGIST.

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The overall objective of the symposium is to foster career development of a toxicologist by providing education and knowledge about different facets of a toxicology career. The goals of this session are: 1. To provide insights into various facets of a successful career in toxicology. 2. To highlight qualities necessary to develop these career characteristics. 3. To provide a path forward towards developing a career as an academic, industrial or entrepreneurial toxicologist. This session will have three parallel components. Firstly, it will be targeted towards the career development of early career toxicologists including graduate students, postdoctoral fellows and young investigators who are at the crossroads of deciding a suitable path forward in toxicological sciences. The presentation will give an in-depth perspective about career as an industrial toxicologist, toxicologist in drug development, academic toxicologist, clinical toxicologist, forensic toxicologist, ecotoxicologist, regulatory toxicologist, or occupational toxicologist. The second component is targeted towards educating the toxicologists about developing adequate skill set necessary to manage a laboratory in academics or in industry and secondly towards applying for potential funding opportunities to support the laboratory. The third component is geared towards an entrepreneurial toxicologist desiring to start a company at any stage of his/her career. This presentation will lay out different steps involved from having the initial idea to starting a company, including approaching the venture capitalist, strategy design, implementation and execution, etc.

IS 665 CAREERS IN TOXICOLOGY.

J. Popp. *Stratoxon LLC, Lancaster, PA.*

Toxicology will continue to provide rewarding career opportunities in the future. However, all current and future toxicologists will need to adapt to the rapidly changing world to fully take advantage of the future opportunities. Changes affecting toxicology include technological advancements, greater interaction of scientific disciplines to address medical and environmental issues and integration of scientific activities across international borders. Toxicologists must be able to utilize new technologies (e.g. the omics) to solve problems rather than focusing on the technology. The future demands interaction of scientific disciplines since problems are now too complex for individual resolution, funding sources are demanding results that can not be achieved individually and there is an increased expectation for science to meet societal needs. These trends are all expected to continue and intensify in the future. Integration of activities across administrative boundaries at all levels including international political boundaries is imperative for success in the future of science in general including toxicology. International integration of business activities is far advanced compared to integration of scientific activities including toxicology. However, we are now seeing rapid expansion of integration of scientific activities in many areas including toxicology in some cases driven by "outsourcing" of science to other countries. To take advantage of opportunities, the successful toxicologist of the future must be very adaptable in both the technological arena and interaction within and outside of the scientific community.

IS 666 MANAGING A LABORATORY.

V. McGovern. *Burroughs Wellcome Fund, Research Triangle Park, NC.* Sponsor: V. Vaidya.

There are many big questions ahead for those who are about to start their own labs: with what kind of workers—technicians? postdocs? grad students?—will you staff it? What will your strategy be for equipping your lab, and how can you spend your materials/equipment/supplies/disposables budget efficiently? How will you build your funding, lay out a plan for the first few years, and get on good footing for pursuing tenure? Insights from the Burroughs Wellcome Fund's experiences in helping hundreds of postdocs get started will illuminate a lively discussion of how to prepare yourself for the big decisions faced immediately by brand new faculty members. Session attendees will go home equipped with some guidelines for thought experiments that will help them sketch out their start-up needs, funding possibilities, and what they will require from their first few hires.

IS 667 NIH GRANT OPPORTUNITIES IN TOXICOLOGY.

P. J. Mastin. *NIEHS, Research Triangle Park, NC.* Sponsor: V. Vaidya.

The National Institutes of Health (NIH) offers numerous options for funding toxicology-related research, for investigators in academics as well as those in small businesses. This presentation will provide information on the overall NIH extramural grants process and on the types of grants that are available to researchers. In addition to the standard R01, information on grants that may be more suited for researchers in the early stages of their careers and ones directed specifically toward post-doctoral fellows and early-career faculty will be discussed, along with information on Small Business grants. Examples of funding opportunities that are available at the time of the meeting will also be presented. Suggestions on improving your chances for funding will also be considered, along with resources for investigators who are preparing applications.

IS 668 STARTING A COMPANY.

S. B. Farr. *Vista Therapeutics, Santa Fe, NM.* Sponsor: V. Vaidya.

Starting a company in the field of toxicology is unlike starting almost any other type of enterprise for one simple reason. The majority of money spent on drug safety is in support of IND and NDA applications where the assays and techniques used are highly proscribed by national and international guideline and regulations. The harsh reality is that most toxicology is performed to fulfill regulatory obligations, and not to deeply explore the true toxicological potential of a candidate compound. Innovation is not rewarded in toxicology as it is in most other fields. For instance, the molecular biology revolution wherein molecular responses to adverse stimuli provide mechanistic insights impossible to obtain otherwise has only recently forced itself upon drug companies and the regulatory communities. Current advances in personalized risk assessment will force themselves into the field of regulatory toxicology. This talk will focus on how to understand and take advantage of the unique characteristics and dynamics of the toxicology market. It will also discuss some of the nuts and bolts considerations in building a fundable new enterprise in the field of toxicology.

669 A NOVEL METHOD FOR DERIVING MECHANISTICALLY-ANCHORED GENE EXPRESSION BIOMARKERS.

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Gene expression biomarkers predictive of toxic or pharmacologic endpoints are a useful component of the molecular toxicology toolbox, and are being applied in preclinical drug development to rank and prioritize compounds. The FDA is paying close attention to the utility and application of gene signatures in safety assessment and clinical settings through the MAQC consortium. An ideal biomarker is

accurate, robust and biologically-meaningful. Current approaches to deriving genomic biomarkers can produce reasonably accurate biomarkers, but these lack robustness (highly dependent on platform and experimental design) and cannot generally be linked biologically to the endpoint. One barrier to the more extensive use of these genomic biomarkers is the difficulty in determining the biological relevance of the signatures from the classifying genes identified, limiting their utility for risk assessment. We have developed a novel method for deriving genomic signatures using discrete modules of genes representing a variety of biological pathways and functional categories, and two-stage machine-learning approach that identifies individual modules with classification power, and combines them into a meta-signature to optimize predictive performance. These "functional descriptors" have comparable performance to gene signatures for the same endpoint generated using other supervised machine learning methods. A functional descriptor predicting renal tubule injury was derived with an estimated sensitivity of 87.5% and specificity of 96.8%, comparable to the performance of a standard gene signature on the same training set (83% and 94% respectively). Functional descriptors also encompass information about the pathways and metabolic processes involved, leading to an understanding of the biological relevance of the signature. Classification of tubule toxicity was based on perturbation of pathways involved in cytoskeletal remodeling, lipid metabolism, vitamin D signaling and amino acid metabolism amongst others. Functional descriptors therefore hold the promise of combining predictive and mechanistic systems toxicology.

670 INDEPENDENTLY PUBLIC TOXICOGENOMICS STUDIES VALIDATE THE TRANSSULFUATION PATHWAY AS POTENTIAL LIVER TOXICITY PATHWAY.

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DNA microarrays and metabolomics can be used to investigate the biological processes involved in disease and toxicity through an integrated analysis of gene expression and metabolc networking. Such an integrated approach is expected to result in a better understanding of this complex biological process. In a previous 1D 1H NMR-based metabolomics study, we identified N-methylnicotinate, involved in SAM transsulfuration pathway, as a potential toxicity biomarker by analysis of the metabolomics data from 14 liver toxicity compounds. In this study, two public gene expression datasets from the Gene Expression Omnibus (GEO) database were used to validate the effect of liver toxicants on the SAM transsulfuration pathway to produce glutathione. In one gene expression study (GEO entry GSE2303), examination the gene expression in rat liver responding to treatment of three prototypical liver toxicants (i.e., Clofibrate, DEHP, VPA), that genes mat1a, ahcy, bhmt, cbs and tat involved in the SAM transsulfuration pathway were found to be significantly altered. In another study (GEO entry GSE5909), the results indicated that, similar to Study 1, a set of the SAM transsulfuration pathway related genes (e.g., mat1a, cbs, ahcy, and mtr) were significantly altered in the 3 liver-toxic group, but not in the 3 non-liver-toxic group. Therefore, our analysis of independent, public genomics data substantiates findings from our previous metabolomic study: that the SAM transsulfuration pathway involved in glutathione production is affected by known liver toxicants. Consequently, urinary levels of N-methylnicotinate related to the SAM transsulfuration pathway may serve as a noninvasive biomarker of liver toxicity.

671 PHENOTYPIC ANCHORING OF CARCINOGEN-INDUCED GENE EXPRESSION REVEALS A COINCIDENCE BETWEEN THE NO TRANSCPTIONAL EFFECT LEVEL (NOTEL) AND THE NO DETECTABLE ADDUCT LEVEL (NODAL): IMPLICATIONS FOR RISK ASSESSMENT?

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We used a toxicogenomics approach to explore dose dependent transcriptional responses to carcinogens in vitro and in vivo, and examined how transcrptional responses were related to the formation of genotoxic DNA adducts. For in vitro studies, logarithmically growing human BEAS-2B bronchial epithelial cells were dosed for 24 hours with the active metabolite of PhIP (N-hydroxy-PhIP) at concentrations ranging from 10^{-5} to 10^{-11} M. Total RNA and DNA were simultaneously isolated from each exposed cultures. RNA preparations were used to generate cRNAs and hybridized to Affymetrix GeneChip Human Genome U133 Plus arrays, and data were extracted and analyzed for changes in gene expression as a function of carcinogen dose. For measurements of adduct formation, genomic DNA was digested to mononucleosides and the adducted bases enriched by solid phase extraction. Samples were reconstituted in 20 ul deionized water and 5 ul was injected. DNA adduct levels were quantitated using an Agilent ion trap MS with HPLC Chip Cube interface. The calibration curve, with a stable isotope d3-PhIP-dG internal standard, was linear over the range of 0.1-10 fmol/ul with an R2 of 0.995. The limit of detection for PhIP-dG adducts was 0.04 fmol/ul. Using this system, adducts were detected in the dosing range of 10^{-5} to 10^{-8} M N-hydroxy-PhIP concentrations. Significantly, doses of PhIP that failed to yield detectable levels of adducts (NODAL) corresponded closely to the doses that had no observable effect on the cell's gene expression profile (NOTEL). Similar in vivo experiments using rats exposed to deceasing, non-tumorigenic doses are in progress. These finding could have significant implications for the use of default assumptions in extrapolating dose during risk assessment for genotoxic compounds and carcinogens.

672 TRANSCRIPT PROFILING REVEALS DIVERGENT REGULATION OF MITOCHONDRIAL METABOLISM IN HUMAN AND MOUSE MODELS IN RESPONSE TO XENOBIOTICS.

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Although rodent models are often used to measure potential xenobiotic toxicity, it is unclear how representative these models are for humans. To this end, transcript profiling in primary human hepatocytes from ten donors and in liver tissues from C57BL/6 mice was performed after a 24 hour treatment with two doses of three xenobiotics: Aroclor 1254, an environmental contaminant; phenobarbital, an enzyme-inducing barbiturate drug; and di(2-ethylhexyl) phthalate, a plasticizer. ~12000 orthologous genes on human and mouse genome arrays were identified using the NetAffx Analysis Center (Affymetrix). Using only these genes, <8% of the genes changed in any one human hepatocyte donor were similarly regulated in a mouse receiving comparable treatment. Of the three chemicals, Aroclor 1254 elicited the greatest number of changes conserved across species. Genes related to oxidative stress response, chaperone activity, and inhibition of apoptosis were increased and genes related to cell cycle control and induction of apoptosis were decreased, consistent with reported generation of reactive oxygen species and promotion of cell growth by Aroclor 1254. In mice, Aroclor 1254 overwhelmingly lowers expression of genes involved both in the TCA cycle, such as citrate synthase and isocitrate dehydrogenase, and in all complexes of the mitochondrial respiratory chain. This reduction in mitochondrial metabolism is likely a secondary effect due to increased oxidative stress, ultimately leading to an inhibition of ATP synthesis and contributing to the toxic effects of Aroclor 1254. Interestingly, in human hepatocytes, many of these genes are predominantly increased. Species-specific regulation of mitochondrial metabolism may mechanistically underlie differential susceptibility to toxic effects of Aroclor 1254 in mice and humans. Supported by NIEHS, ES11387 and NIGMS, GM66411.

673 DIFFERENCES IN GENE EXPRESSION PROFILES FROM TCDD-, 4-PECDF- OR TCDF-TREATED PRIMARY RAT AND HUMAN HEPATOCYTES.

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Remarkable differences exist in the species sensitivities and biochemical and toxic effects induced from exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD or dioxin) and dioxin-like chemicals. For example, at least an order of magnitude difference has been reported for TCDD-induced CYP1A1 mRNA and EROD activity in rats compared to lower sensitive humans with this response being one of the most, if not the most, significant adaptive responses to TCDD-AHR activation. To better understand the lower sensitivity of humans, primary human and Sprague-Dawley rat hepatocytes (adult female; n=5/species) were used as models to research species- and congener-specific differences in gene expression profiles after 24 hours treatment with TCDD, 2,3,4,7,8-pentachlorodibenzofuran (4-PeCDF), or 2,3,7,8-tetrachlorodibenzofuran (TCDF) at the EC50's for CYP1A1 mRNA induction. The results indicate that at equipotent concentrations significantly more

genes (>2X) were altered in rat cells compared to human cells and there was little overlap between the genes altered by TCDD when compared to those altered by the furans. Moreover, only a very small number of common genes were altered between rat and human cells treated with equipotent concentrations of TCDD (5 genes), TCDF (4 genes) or 4-PeCDF (4 genes) and these consisted largely of well characterized AHR core battery genes. These results indicate that (1) TCDD and furans induce significantly different gene expression patterns in both humans and rats, and (2) significant differences exist in the response of rats vs. humans to TCDD and other dioxin-like chemicals. Whether or not these gene expression differences can explain the great difference between humans and rats in response to TCDD-induced AHR activation has yet to be determined. However, these differences in gene expression could be significant factors when attempting to extrapolate rat data to human risk characterization.

674 COMPARATIVE METABOLOMIC ANALYSIS OF HEPATOTOXICITY EXPERIMENTS IN RATS AND MICE.

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We have previously reported a data processing and analysis methodology for Nuclear Magnetic Resonance (NMR) spectrometry that locally aligns spectra, allowing comparative studies in which effects due to anomalous chemical shifts resulting from sample environmental factors are minimized. Principal Component Analysis (PCA) is used to perform pattern recognition and identify data clusters. We have extended the methodology by using principal components to filter the data and emphasize the spectral peaks that are most active in driving the clustering ("PCA filtering"). Using this methodology, the 13C NMR metabolomic responses to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), referenced to vehicle-treated controls, were examined in immature ovariectomized C57BL/6 mice and Sprague-Dawley rats, to further investigate species-specific hepatotoxicity. Single-species PCA found a prominent treatment effect in mice (well-separated control and treatment clusters in a PCA scatterplot) and a weaker effect in rats (significant overlap of controls and treatments). The chemical association of peaks identified by the PCA filtering technique indicated that TCDD treatment in mice is characterized by the redistribution of unsaturated fatty acids, cholesterols, and triacylglycerols. The corresponding analysis of rat data showed no statistically significant individual peak changes with treatment. PCA of the combined data from both species revealed three clusters in a PCA scatterplot corresponding to mouse controls, mouse treatments, and undifferentiated rat data. Funded by NIH/NIEHS RO1 ES013927.

675 APPLICATION OF A SYSTEMS TOXICOLOGY APPROACH TO INVESTIGATE TROGLITAZONE HEPATOTOXICITY IN THE RAT.

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Troglitazone (TGZ), a PPAR-γ agonist oral antidiabetic agent, was withdrawn from the market due to severe idiosyncratic hepatocellular toxicity in man. In the scope of the Innomed PredTox project, TGZ was tested as a model hepatotoxic compound using an integrated approach combining genomics, proteomics and metabonomics, along with conventional toxicology evaluation. TGZ was administered orally to male Wistar rats (n=5) at doses of 0, 200 or 1500 mg/kg/day for 1, 3 and 14 days. After a 14-day administration increased liver weights and a mild centrolobular hypertrophy at 1500 mg/kg/day were observed in all five animals. Gene expression analysis revealed a strong impact of high doses of TGZ on the liver transcriptome, peaking on Day 2 and decreasing with longer treatment duration. Functional analysis mainly indicated the pharmacological action of TGZ, namely induction of lipogenesis, inhibition of glucose production/utilization, activation of glycogenesis and repression of the inflammatory response. TGZ also presented PPAR-α activity at high doses: enhanced fatty acid β-oxidation, tightly coupled to induction of Krebs cycle and ketogenesis. Detoxifying enzymes were also induced, most likely underlying the centrolobular hypertrophy observed microscopically. Functional annotation of the 80 modulated proteins identified by 2D-DIGE confirmed the mobilization of lipid metabolism and glycolysis inhibition. Induction of mitochondrial and protein metabolisms, and a cellular stress response were also observed. Urine NMR analysis revealed an initial up-regulation of fatty acid metabolites. Trimethylamin-N-oxide, a chemical chaperone linked to protein metabolism, and betaine were up-regulated in a time-dependent manner. Integration the three "omics" approaches allowed the identification of the most relevant pathways involved in the toxicity of TGZ.

676 INTEGRATED TRANSCRIPTOMIC AND PROTEOMIC EVALUATION OF GENTAMICIN NEPHROTOXICITY IN RATS.

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Gentamicin is an aminoglycoside antibiotic, which induces renal tubular necrosis in rats. In the context of the InnoMed PredTox project (www.innomed-predtox.com), transcriptomic and proteomic studies were performed to provide new insights into the molecular mechanisms of gentamicin-induced nephrotoxicity. Male Wistar rats (n=5 per group) were treated with 25 and 75 mg/kg/day subcutaneously for 1, 3 and 14 days. Gene expression changes in kidney and blood were assessed using Affymetrix RAE230plus microarrays and protein modulations were evaluated by two dimensional difference gel electrophoresis (2D-DIGE) technology followed by mass spectrometry identification. Analysis of genomic data indicated a strong treatment-related gene expression modulation in kidney and blood at the high dose after 14 days of treatment, with the regulation of 463 (303 up and 160 down) and 3246 (923 up and 2323 down) genes respectively. The modulated genes were involved in the activation of the p38 MAPK cascade, NF-kB pathway, inflammation, apoptosis, protein metabolism, cell proliferation and T-cell activation. It is noteworthy that the top up-regulated gene in kidney was the nephrotoxicity biomarker KIM-1. The proteomic study showed an up-regulation of 49 proteins and a down-regulation of 57 proteins in kidney with 75 mg/kg/day after 14 days of treatment. Proteomic results were suggestive of a mitochondrial dysfunction with impairment of cellular energy production, an induction of oxidative stress, an effect on protein biosynthesis and on cellular assembly and organization. Transcriptomic and proteomic data turned out to be complementary and their integration gave a more comprehensive insight into the putative mode of nephrotoxicity of gentamicin that was in accordance with the tubular necrosis/regeneration observed in histopathology.

677 TOXIGENIC PHASE OF ANTHRAX INFECTION: BIOLOGICALLY-BASED MODELING OF MODULATION OF MAP KINASE SIGNALING PATHWAY.

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Anthrax (bacillus anthracis) owes its pathogenicity to two determinants of virulence: the formation of a poly-D-glutamyl capsule, which mediates the invasive (proliferation) stage of the infection as it reproduces inside macrophages and other immune cells, and the production of the multicomponent anthrax toxin which mediates the toxigenic (terminal) stage. One component, lethal factor (LF), cleaves most isoforms of mitogen-activated protein kinase kinases (MAPKKs) close to their N-terminus. This interferes with a major signaling pathway linking the activation of membrane receptors, such as TLR4, to the transcription of several genes, including those encoding pro-inflammatory cytokines, as well as the p38 MAPK dependent expression of pro-survival genes. To develop models for the toxigenic phase involving interaction of lethal factor with host cells, existing parameterized models of the MAPK pathway in SBML (Systems Biology Markup Language) were translated into Berkeley Madonna™, a format suitable for integrated physiologically-based modeling of both anthrax proliferation and host system response. Cleaving the active and inactive isoforms of MAPKK (beyond the ability of the host to replenish them) by LF leads to a decline (to zero) in a MAPK model demonstrating response ultrasensitivity (Huang, 1996) and in a MAPK model showing the oscillatory signaling output (Kholodenko, 2000) that has a characteristic half-time that can be correlated with the net potency of the toxin. When combined with the proliferation model that simulates LF dosimetry, and with a description of the biological effect of signal reduction in terms of the cellular response (alterations in gene expression

leading to changes in cytokine production, apoptosis etc.), we have a model framework that predicts the time-course of the biological effects of anthrax in biologically varied individuals.

678 BIOLOGICAL AND MOLECULAR MARKERS FOR SULFUR MUSTARD ANALOG CEES-INDUCED SKIN INJURY IN MOUSE AND HUMAN EPIDERMAL KERATINOCYTES.

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Sulfur mustard (HD) is a cytotoxic and alkylating chemical warfare agent that has its primary effect on skin. Despite various studies in literature, established skin models are still needed to develop a comprehensive approach towards identifying biological and molecular markers of HD-induced skin injuries. The central focus of our study was to identify and establish the molecular and biological markers for HD-induced skin injury employing mouse epidermal JB6 and human epidermal HaCaT keratinocytes as accelerated model systems, and to further apply these to agent efficacy studies. We evaluated the effects of 2-chloroethyl ethylsulfide (CEES) on cell viability (MTT assay), cell proliferation/DNA synthesis (BrdU assay), and apoptosis (caspase-3 activity assay and Hoechst/PI staining) in these cells. A decrease in cell viability and DNA synthesis was observed with increasing concentrations of 0.1-1mM CEES within 2h of its treatment with more pronounced effects at 4 and 24h. Apoptosis was observed within 4h of CEES treatments in HaCaT cells, whereas it was observed only after 24h of CEES treatments in JB6 cells. This was further confirmed by western blot analysis of apoptosis markers, PARP and caspase-3. Cell cycle analysis showed S-phase arrest after CEES treatments in these cells which lasted up to 24h where an increase in G2/M arrest of cells was also observed. Consistent with above results, molecular marker studies using western blot analysis showed phosphorylation of H2A.X at ser139 and of p53 at ser15. Phosphorylation of ATM/ATR protein kinases, cell cycle proteins chk1 and chk2, cdc25C, cdc2 and accumulation of cyclin B as well as mitotic arrest of cells was also observed supporting cell cycle results. Together, our studies establish models and biomarkers of CEES-induced skin injury, which would be useful for agent efficacy studies towards developing effective medical countermeasures for sulfur mustard toxicity.

679 NANOPARTICLE-BASED ELECTROCHEMICAL IMMUNOSENSOR FOR DETECTION OF PHOSPHORYLATED ACETYLCHOLINESTERASE ADDUCT: AN EXPOSURE BIOMARKER OF ORGANOPHOSPHATE PESTICIDES AND NERVE AGENTS.

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Simple, rapid and sensitive sensors are needed to evaluate chemical nerve agent exposures. To detect sub-clinical exposures, sensor development is focused on the detection of adducts on target proteins. Specifically the development of an electrochemical immunosensor for determination of phosphorylated cholinesterase (OP-ChE) adduct biomarkers using quantum dot (QD) labels. Paraoxon was used as a model organophosphrous compound to prepare phosphorylated acetylcholinesterase (AChE) to demonstrate the proof of principle. A nanoparticle-based electrochemical immunosensor has been developed for the detection of OP-ChE. Zirconia nanoparticles (ZrO_2 NPs) were used as selective sorbents to capture the phosphorylated AChE adduct, and quantum dots (ZnS@CdS, QDs) were used as tags to label monoclonal anti-AChE antibody to track the immunorecognition events. The sandwich-like immunoreactions were performed among the ZrO_2 NPs, which were pre-coated on a screen printed electrode (SPE) by electrodeposition, phosphorylated AChE and QD-anti-AChE. The captured QD tags were determined on the SPE by electrochemical stripping analysis of its metallic component (cadmium) after an acid-dissolution step. The paraoxon-AChE adduct was characterized by Fourier Transform Infrared Spectroscopy (FTIR) and the binding affinity of anti-AChE to the paraoxon-AChE was validated with an enzyme-linked immunosorbent assay. The parameters (e.g., amount of ZrO_2 NP, QD-anti-AChE concentration,) that govern the electrochemical response of immunosensors were optimized. The voltammetric response of the immunosensor is highly linear over the range of 10 pM to 10 nM paraoxon-AChE, and the limit of detection is estimated to be 8 pM. This new nanoparticle-based electrochemical immunosensor thus provides a sensitive and quantitative tool for biomonitoring exposure to OP insecticides and chemical nerve agents. (Supported by NIH/NINDS grant NS058161-01)

680 TRANSCRIPTIONAL RESPONSES IN PORCINE SKIN FOLLOWING SULFUR MUSTARD AND THERMAL BURNS.

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Sulfur mustard (HD) and thermal injuries represent military and civilian threats, resulting in significant skin damage. Although similar therapies are used for treating cutaneous thermal and HD injuries, the underlying molecular mechanisms of tissue damage and wound healing may differ between the types of damage. This study utilized microarray analysis to evaluate transcriptional changes in porcine skin to assess potential similarities and differences in the underlying molecular mechanisms between HD and thermal injury. Four ventral abdominal sites on each of four female, Yorkshire crossbred weanling swine were exposed to 400 µL undiluted HD or heated brass rod (70°C) for 8 min and 45-60 sec, respectively. At 48 hr post-exposure, skin samples were excised, total RNA was isolated, processed and hybridized to Affymetrix GeneChip® Porcine Genome Arrays (containing 20,201 genes). Expression analysis revealed that HD and thermal exposure promoted significant changes in transcription where 293 and 268 transcripts were increased with HD and thermal exposure, respectively. HD and thermal exposure promoted decreases in 197 and 718 transcripts, respectively. HD- and thermal-injured skin possessed 150 increased and 148 decreased transcripts in common. Gene ontological analysis showed that HD promoted significant changes in genes associated with catabolism and morphogenesis, while thermal injury promoted significant changes in genes associated with development, cell differentiation, and embryonic development. Both HD and thermal injuries shared molecular functions associated with protein/receptor binding and the cell component categories of extracellular region/space. These results indicate that transcriptional changes can differentiate between thermal and HD cutaneous exposure, which can be useful in developing therapeutics for each type of skin injury. Supported by DTRA/CBMS/MRMC Contract W81XWH-05-D-0001, Task Order 0004 and does not necessarily reflect DoD policy.

681 GENE EXPRESSION PROFILING OF PORCINE SKIN EXPOSED TO SULFUR MUSTARD.

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Bis-(2-chlorethyl) sulfide (sulfur mustard, SM) is a potent alkylating agent that induces cutaneous vesication in humans. The molecular mechanism of cutaneous vesication is not completely understood, and the molecular pathways involved in post-exposure wound healing are not well characterized. To elucidate these molecular pathways, we used oligonucleotide microarrays to identify gene expression profile changes induced by SM in porcine skin, an established animal model of SM exposure and wound healing. Female Yorkshire crossbred pigs were exposed on the ventral surface to neat SM for eight minutes using an exposure template to obtain uniform skin lesions. Skin punch biopsies were collected at one hour, 24 hours, or seven days post-exposure. RNA extracted from the biopsies was used to generate probes for porcine genome microarrays. Principal component analysis revealed that the main sources of variability were treatment and time. From a two-way analysis of variance based on the factors of treatment and time, a list of ~590 significant genes was obtained ($p<0.0001$). Although the porcine model is an established animal model, we estimate that less than 20% of the pig genome is currently annotated. This lack of annotation poses a challenge for identifying specific genes involved in the molecular toxicity of SM. To address this, unannotated target gene sequences were aligned to the Sus scrofa, Bos taurus, Mus musculus and Homo sapiens genome databases using the NCBI nucleotide Basic Local Alignment Search Tool (BLAST N). From these alignments, gene homology was inferred for target sequences across various species, with specific identified genes representing the biological processes of cell cycle regulation, inflammation, and apoptosis. Comparison of these gene expression profiles with histopathology data correlate the gene expression profiles with phenotypic responses. This work provides important data for the development of therapeutic strategies to treat cutaneous SM exposure.

682 REAL-TIME PCR QUANTITATION OF SINGLE-BASE SUBSTITUTIONS IN RNA, AND ITS APPLICATION TO THE DETECTION OF RICIN AND OTHER RIBOSOME-INACTIVING PROTEINS.

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Ricin, a highly toxic "ribosome inactivating protein", kills cells by removing a specific adenine from the elongation factor binding site of 28S ribosomal RNA, thus inactivating ribosomes. We have adapted a property of reverse transcriptase to create a simple, sensitive assay for this potential bioterrorism agent. We show that

Moloney murine leukemia reverse transcriptase preferentially inserts an adenosine (A) opposite an abasic site in RNA, in common with DNA-dependent DNA polymerase's action on DNA with damaged sites; this produces cDNA with an A opposite the abasic site. By designing primers to detect both the native and the altered sequences, we can quantitate the change with quantitative real-time PCR (qPCR). For the ricin assay, reverse transcription of the RNA leads to an A-to-T transversion in the cDNA at toxin-modifed sites and qPCR quantitates the depurination. This assay has been used to determine an optimum pH of about 5 for the ricin reaction with RNA. Ricin activity is undiminished by the presence of 0.5% Triton–X100 or Tween 80, a fact which should facilitate recovery of ricin from contaminated surfaces.
The most commonly used in vitro assay for ricin uses antibodies to detect its presence but not its activity. Cell culture and animal assays also detect toxin activity, but are lengthier and more tedious; and animal assays submit the animals to unnecessary suffering. A feature of ricin which increases its toxicity is the presence in the toxin of a second protein chain which facilitates its entry into the cell. Neither the immunoassay nor the qPCR assay as described above detects this activity. However, the qPCR assay can measure the damage to total RNA isolated from ricin-treated cell cultures, providing confirmation, if needed, of that aspect of the toxin's activity. Because our new assay measures the protein's enzymatic activity, it should also be useful for detecting other toxins that depurinate 28S rRNA, such as abrin, a potential bioterrorism agent, and shiga toxin, the active agent in the dangerous food contaminant E. coli O157:H7.

683 DIFFERENTIAL GENE EXPRESSION IN SULFUR MUSTARD TREATED MURINE SKIN AFTER TREATMENT WITH MMP-2/MMP-9 INHIBITOR I.

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Sulfur mustard (SM)causes blistering of skin after several hours. A potential countermeasure agent to SM-induced injury, MMP-2/MMP-9 Inhibitor I, was used in a time course study to evaluate its therapeutic effectiveness and identify subtle gene changes by microarray analysis. Punch biopsies of mouse ears exposed to 0.08 mg SM with and without inhibitor were examined histologically and assayed for gene expression profiling by Affymetric microarray analysis. Principal component followed by ANOVA analysis with a false discovery rate of 0.05 was used to create a gene profile list. KEGG library pathways analysis for each time period was performed and the data assigned Fisher p values. Common pathways between the SM + inhibitor and SM alone groups included: apoptosis, immune system and signal transduction pathways for 24h, cytokine-cytokine receptor interaction and various metabolic pathways for 72h, and wound repair pathways for 168h. The most significant difference was a reduction in oxidative phosphorylation for 72h (more than 20 oxidative stress-related genes down regulated). The Welsh T test ($p < 0.05$) was used to directly compare the effect of the inhibitor on SM exposure and identified over 1000 (24h), 2000 (72h), and 1100 (168h)genes that met the criteria for either up or down regulation. Further analysis identified the major upregulated pathways at 24 h as biosynthesis and metabolism related, at 72 h as cell cycle and regulatory pathways, and at 168h as wound repair pathways. Our results suggest that subtle gene changes by microarrays can be used to screen potential therapeutic countermeasures against sulfur mustard skin injury. This work is supported by ES05022, ES07148 and the NIH CounterAct Program NIAMS U54AR055073. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the federal government.

684 BIODEGRADABLE MICROSPHERE DELIVERY SYSTEM FOR BUTYRYLCHOLINESTERASE TO PROTECT AGAINST ORGANOPHOSPHATE TOXICITY.

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Human butyrylcholinesterase (HuBChE) is advancing for regulatory approval as prophylactic treatment for protection against chemical warfare nerve agents (CWNAs). However, it has several amino acid variations which may elicit immune responses if repeatedly administered to soldiers. Also, it has a half-life of 8-12 days in humans, and a 3-5 day pretreatment is necessary to protect against twice the median lethal dose in humans. To increase the retention and reduce the immunogenicity, we encapsulated BChE in biodegradable D,L poly(lactid-co-glycolide) (PLGA) microspheres. PLGA is FDA approved for use in humans, and its degradation is preceded by the influx of water. This creates an ideal environment for bringing the CWNAs to the enzyme. We demonstrate here that BChE retains its activity in the presence of the compounds used to encapsulate BChE in PLGA microspheres (dichloromethane, PLGA, and polyvinyl alcohol). Furthermore, we evaluated different ratios and viscosities of PLGA for encapsulation efficiency and effectiveness for inhibiting organophosphates (OPs). We determined the enzyme activities with an Ellman assay, amount of enzyme encapsulated using a bicinchoninic acid (BCA) protein assay, and the amount of diisopropylfluorophosphate (DFP) the BChE microsphere formulations inhibit using an AChE backtitration Ellman assay. Overall, we found the 50:50 lactide:glycolide microspheres are much more effective than 75:25, and that the high viscosity 50:50 is more effective in encapsulating the enzyme than the lower viscosity and seemed to release at a slower rate. A 50:50 formulation with a viscosity ranging from 0.55-0.75, was found to be the best candidate for OP inhibition. These formulations can significantly reduce health care costs by reducing the number of injections required and possible immune responses. The opinions and assertions contained herein are the private view of the authors and are not to be construed as official or as reflecting the views of the Army, Navy, or the Department of Defense.

685 MODULATION OF OSTEOBLAST DIFFERENTIATION BY A MECHANISM INVOLVING THE ARYL HYDROCARBON RECEPTOR.

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Metabolic bone diseases arise as a result of an imbalance in bone cell activities. Recent evidence suggests that environmental toxicants may be contributing factors altering these activities. We show that AHR expression in osteoblasts parallels the induction of early bone-specific genes involved in maturation. The AHR may not only mediate the effects of toxicants, but with an as yet unidentified ligand, be involved in the differentiation pathways of osteoblasts. AHR deficient mouse bone marrow cells showed reduced differentiation to mature nodule forming osteoblasts compared to wildtype mice. Moreover, the significant reduction in alkaline phosphatase activity by differentiating AHR deficient osteoblasts indicates that a delicate balance of AHR activity is required for nodule formation. AHR knock out mice bone marrow cells showed a 70% reduction in bone nodule formation after 14 and 21 days of culture compared to wildtype. These findings demonstrate that AHR is required for optimal alkaline phosphatase activity in differentiating osteoblasts. Furthermore, lead treatment of osteoblasts upregulates the expression of AHR mRNA and protein levels supporting a novel mechanism whereby lead in the skeleton may increase the sensitivity of bone cells to toxicant exposure. Given that AHR expression peaks during ostetoblast differentiation, this critical stage of bone development should be considered a susceptible target for toxic ligands to disrupt normal bone formation processes. The ability of lead to actively alter bone processes immediately after it becomes sequestered in the skeleton and throughout the life of an organism is appreciated, but still not yet well-understood. The likelihood of successive and continuous exposure to organic and inorganic compounds in human populations supports the need for future studies to address the effects of mixtures, particularly because bone tissue is a reservoir for lead.

686 NEW INSIGHTS INTO HOW SUSTAINED AHR ACTIVATION DURING DEVELOPMENT CAUSES LONG-LASTING ALTERATIONS IN IMMUNE FUNCTION.

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There is growing evidence that environmental factors influence immune system development and programming. However, the mechanisms by which they do so are not clear. One important modulator of immune development is the aryl hydrocarbon receptor (AhR). We have previously shown that developmental exposure to the AhR agonist TCDD yields adult offspring with defects in their response to influenza virus. Alterations include suppressed lymphocyte responses and increased inflammation. The present studies were conducted to determine the critical period during immune ontogeny that is particularly sensitive to inappropriate AhR activation. We then investigated the contribution of AhR-mediated events within and extrinsic to hematopoietic cells by creating reciprocal bone marrow chimeras. We show that sustained AhR activation altered different elements of the response to infection at different times during development by affecting different tissue targets. Specifically, diminished T cell responses arise due to deregulated events within bone marrow derived cells. In contrast, increased IFN-gamma production by phagocytic cells results from AhR-regulated events extrinsic to leukocytes, and requires AhR activation during early gestation. In separate studies, immune modulation was compared in exposed dams and their adult offspring, revealing that AhR activation causes long-lasting functional alterations in the developing immune system, whereas the impact on the mature immune system was transient. These findings have important implications regarding future studies to delineate how AhR contributes to differences in immune programming. More broadly, they emphasize the

idea that order to fully understand how environmental factors in-play during development contribute to persistent and often subtle changes in immune function, we need to consider effects on non-hematopoietic cells and conduct studies in which functional endpoints form a critical component.

687 IN UTERO AND LACTATIONAL TCDD EXPOSURE ALTERS LIPID METABOLISM IN MALE C57BL/6 MOUSE OFFSPRING FED A HIGH-FAT DIET.

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An increased incidence of obesity in people in western countries has caused a serious concern because the obesity is a contributing factor of metabolic syndrome. Epidemiological and experimental studies report that exposure to 2, 3, 7, 8- tetrachlorodibenzo-*p*-dioxin (TCDD) may be related to an increase in the prevalence of type 2 diabetes mellitus and insulin resistance in humans and in an abnormality in glucose metabolism of lab animals, respectively. Here, we studied the possible aggravation of type 2 diabetes mellitus by *in utero* and lactational exposure to TCDD in mice fed a high-fat diet, with the objective of establishing it as an animal model for obtaining greater insight into the pathogenesis of diabetes. Pregnant C57BL/6 mice were administered either a single oral dose of TCDD (3 μg/kg) or corn oil on gestation day 12.5 and their male pups were reared and weaned. From postnatal day (PND) 28, the male offspring were fed either a regular (R) diet or high-fat (F) diet, generating 4 treatment groups: control-regular diet (C-R), control-high fat diet (C-F), TCDD-regular diet (T-R), and TCDD-high fat diet (T-F). Besides monitoring body weight, diet intake and blood pressure, offspring were examined for abnormalities in glucose or lipid metabolism at 15 or 21 weeks of age. In both control (C-F) and TCDD (T-F) mice fed a high-fat diet, body weight, blood glucose, blood urea nitrogen, and the states of insulin resistance and glucose tolerance were aggravated to a similar extent. Unexpectedly, livers from the T-F group had suppressed the up-regulated mRNA abundance of PPAR-γ, TNF-α, adipophilin, and COX-2 that was observed in the C-F group ($p<0.05$). Elevated expression of genes involved in lipid oxidation and synthesis tended to be decreased by TCDD in the high-fat group (T-F). Thus, in utero and lactational exposure to TCDD appears to affect hepatic gene expression in a way that alters lipid metabolism later in life.

688 OBESITY AND PERINATAL TCDD EXPOSURE INCREASES MAMMARY TUMORS IN FVB MICE.

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Risk of breast cancer has been consistently shown to correlate to total lifetime exposure to estrogens. Because both TCDD exposure and the state of obesity interact with the estrogen pathway, we wanted to investigate how TCDD and obesity interact with mammary cancer susceptibility in a mouse model. Several rat models suggest that prenatal TCDD exposure promotes chemical models of mammary cancer. Therefore, at 12.5 days post conception, we exposed nulliparous FVB females to 1 μg/kg of TCDD. To model diet-induced obesity, the litters were put on high or low fat diet at parturition. Female offspring were exposed to DMBA at post-natal days (PND) 35, 49, 53, and monitored thereafter for tumor development. Lean DMBA-treated FVB without TCDD exposure had the longest tumor latency. Obese DMBA-treated FVB had substantially more mammary tumors than lean FVB. Tumors grew faster among obese litters compared to lean. While no mammary tumors arose in lean litters perinatally exposed to TCDD and a third of unexposed obese litters had mammary tumors, every obese litter perinatally exposed to TCDD had at least one mammary tumor. Among obese mice, exposure to TCDD was associated with a doubled mammary tumors incidence. TCDD was associated with adenomyoepitheliomas, lesions that are rare in DMBA-treated mice. In summary, obesity increases the sensitivity of FVB to DMBA-induced mammary carcinogenesis. Perinatal TCDD exposure appears to further increase the sensitivity of obese FVB. (This abstract does not reflect agency policy.)

689 DEVELOPMENTAL EXPOSURE OF THE MOUSE TO 2, 3, 7, 8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD) INCREASES THE SUSCEPTIBILITY TO HYPERTENSION AND CARDIAC HYPERTROPHY IN ADULTHOOD.

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Exposure of the mouse during development to TCDD significantly reduces fetal heart weight, increases expression of cardiac genes involved in extracellular matrix remodeling, and induces cardiac hypertrophy and bradycardia postnatally. Further, nearly all TCDD-induced changes in fetal cardiac mRNA expression persist into adulthood. Thus, we tested the hypothesis that in utero and lactational TCDD exposure leads to cardiac hypertrophy and an increased susceptibility to dysfunction in adulthood when presented with a second challenge. Time-pregnant C57BL/6 mice were treated with a single oral dose of corn oil or 6.0 μg TCDD/g body weight on gestation day (GD) 14.5 and allowed to deliver. At 3 mos offspring were treated with either a subpressor or pressor dose of angiotensin (Ang) II for 14 d and cardiac hypertrophy and blood pressure were assessed, respectively. Offspring from TCDD-exposed dams exhibited a thicker left ventricle wall in diastole that was increased further by subpressor Ang II, compared to control offspring. In addition, while offspring from TCDD exposed litters were normotensive, pressor Ang II stimulated an earlier onset and greater degree of hypertension, compared to control offspring. This hypertension was reflected by greater increases in systolic blood pressure in TCDD offspring, compared to controls. These results demonstrate that developmental TCDD exposure increases the susceptibility to cardiovascular dysfunction in adulthood when faced with an additional cardiovascular disease risk factor. Supported by ES012335 and HL078914 to MKW and ES012335-0281 to ACA.

690 IDENTIFICATION OF GENES DEVELOPMENTALLY REPROGRAMMED IN THE UTERUS BY NEONATAL EXPOSURE TO XENOESTROGENS.

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Environmental chemical exposures during sensitive windows of fetal development can reprogram target tissue and alter disease susceptibility later in life. Xenoestrogens are a broad class of endocrine disruptors found in our environment that are able to reprogram the female reproductive tract. We have shown that neonatal xenoestrogen exposure reprograms estrogen responsive genes in the myometrium to promote development of uterine leiomyoma. Little is known about the genetic targets for xenoestrogen-induced developmental reprogramming, therefore, we took a global transcriptional profiling approach to identify genes developmentally reprogrammed by xenoestrogens. We identified 171 differentially expressed genes in tumors vs. normal myometrium. A subset of these genes (19) was found to have a predicted or functional estrogen response element, 16 of which were validated as being differentially expressed by real-time PCR analysis. Remarkably, DES reprogrammed 6/16 of the estrogen responsive genes as determined by real-time PCR and immunohistochemistry. We next determined whether other environmentally relevant xenoestrogens, such as genistein (GEN) and bisphenol A (BPA), share the same gene targets and reprogramming profiles as DES. We found that DES caused genes that were both activated and repressed by estrogen to become hyper-inducible by estrogen. GEN caused genes activated by estrogen to become more responsive, but attenuated rather than reversed repression of gene expression by estrogen. In contrast to DES and GEN, BPA caused repression of genes both activated and repressed by estrogen. Therefore, DES, BPA and GEN had unique effects on estrogen-induced gene expression, revealing novel and intrinsic differences between how these endocrine disruptors induce developmental programming in the uterus.

691 DISPARITIES IN LEARNING, MEMORY AND BEHAVIOR AS A RESULT OF ENVIRONMENTAL CONTAMINANT EXPOSURE DURING GESTATION.

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Previous studies have demonstrated that hippocampal and/or cortical synaptic plasticity and learning behavior is significantly impaired in B(a)P and/or TCDD-exposed offspring. These studies have also revealed that brain to body weight ratios are greater in exposed offspring relative to controls indicative of intrauterine growth retardation which has been shown to manifest as low birth weight in offspring. Recent epidemiological studies have identified an effect of prenatal exposure to airborne polycyclic aromatic hydrocarbons on neurodevelopment within the first three years of life among a minority, inner-city cohort of children (Perera et al., 2006). Here we report data obtained from the utilization of a susceptibility-exposure paradigm that provides a point of reference for neurotoxicological studies of environmental contaminant effects on indices of development in general, and on neurodevelopment in particular. Environmental contaminant induced effects on 1) metabolite bioavailability and disposition to critical CNS structures are presented to be causative to decrements in (2) glutamatergic receptor subunit mRNA and

protein expression, (3) sensory cortex cellular responses, and (4) fixed-ratio performance learning behavior. These studies collectively support associations between neurobehavioral deficits and gestational exposure to environmental levels of these contaminants. A molecular-level mechanism of causality is presented. The affected markers of plasticity serve as templates for learning and memory in model systems and from a vulnerability perspective, serve as targets for dysregulation during development in susceptible populations that have been disproportionately exposed to these environmental contaminants.

692 FETAL EXPOSURE TO CIGARETTE SMOKE ALTERS OFFSPRING RESPIRATORY FUNCTION: A MOUSE MODEL.

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Epidemiologic evidence indicates that children born to mothers who smoked during pregnancy are at an increased risk for certain respiratory diseases later in life, including asthma. Thus, studies in mice were undertaken to determine whether: airway responsiveness was altered in juvenile offspring exposed *in utero* to cigarette smoke (CS); allergen sensitization of the dams worsened the effects of CS exposure on offspring airway reactivity; and, differences in pulmonary response to prenatal CS was influenced by sex. Dams either sensitized by two i.p. injections of ovalbumin (Ova) followed by two intranasal challenges (each 7 d apart) or injected only with PBS were subsequently paired (for 4 d) and exposed to either filtered air or mainstream cigarette smoke (MCS) from gestational day 4 to parturition. Total IgE levels in the dams, determined by ELISA with blood collected from survival facial vein bleeding, was determined to assure sensitization. After weaning, airway responsiveness to the non-specific bronchodilator acetylcholine (Ach) was determined by plethysmography in the male and female offspring 5 wk after birth. Offspring of both sexes born to non-atopic mothers proved more sensitive to airway challenge if exposed prenatally to CS (compared to air exposed sex-matched counterparts). Moreover, results demonstrated significant sex differences regardless of CS exposure. Males born to non-atopic dams demonstrated greater airway hyperresponsiveness (AhR) than exposure matched females, while airway reactivity was enhanced in females born to atopic dams. Maternal atopy did not worsen the effects of prenatal smoke exposure on offspring AhR. In fact, CS exposed pups born to atopic dams were significantly less sensitive to Ach challenge than the sex-/age-matched air controls. This study suggests that children exposed prenatally to CS and female offspring born to non-smoking mothers who were atopic during pregnancy may be at increased risk of airway disease later in life. Supported by IFSH 28-B2600-7893.

693 FISH INTAKE GUIDELINES: N-3 FATTY ACID INTAKE AND CONTAMINANT EXPOSURE IN THE KOREAN AND JAPANESE COMMUNITIES.

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Fish consumption advisories are provided nationally to avoid overexposure to various contaminants. As part of the Arsenic Mercury Intake Biometric Study involving the Japanese and Korean communities living in Washington (US), we obtained fish and nutrient intake data and examined hair for mercury levels of study participants. We have used these data to delineate the need for both nutrient and contaminant concerns to be included when providing guidance. To achieve this, we examined the intake of two n-3 long-chain fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), that are found in fish and associated with a variety of beneficial effects. Fifty-five percent of the Japanese participants exceeded the Reference dose (RfD) due to hair-Hg levels above 1.2 ppm; however, the 95% of those above the RfD obtained their dietary requirement for DHA. The Korean community had only a small percentage of individuals exceeding the RfD (14%) yet had 20% not obtaining their dietary recommendation for DHA. These results indicate that guidance on fish consumption without considering nutritional benefit can lead to dietary behaviors that contradict to dietary recommendations. When quantitatively deriving health guidance from mercury exposure levels, we conclude that the benefits of fish consumption need to be incorporated, such as using DHA and DHA+EPA intake levels. [This work is supported by the US Environmental Protection Agency, Region 10 (Clean Water Act, 104 (b)(3): 66-463), PNW Center for Human Health and Ocean Sciences (NIH/National Institute of Environmental Health: P50 ES012762 and National Science Foundation: OCE-0434087)]

694 SAMPLE SELECTION PROCESS FOR USDA/FSIS NATIONAL RESIDUE PROGRAM.

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The National Residue Program Scheduled Sampling Plan (NRPSSP), United States Department of Agriculture, Food Safety Inspection Service (USDA/FSIS) is a risk based, resource driven multi-component analytical testing program for residue prevention and control of veterinary drugs and pesticides in domestic and imported meat, poultry, and egg products. The range of chemical compounds in the NRPSSP is flexible but comprehensive. Human health risk is a major criterion for selection and prioritizing chemical compounds to be tested under the NRPSSP. In support of the plan, a chemical risk profile (CRP) for each potential chemical has been developed. The components of the CRP include extent of usage, previous violations, established regulatory limits, acceptable daily intake/reference dose, no observed adverse effect level (NOAEL), LD50, chronic toxicity or other adverse clinical signs such as anaphylactic reactions, development of antibiotic resistance in microorganisms, public concern, and the concerns of the National Antibiotic Resistance Monitoring System, the Food Animal Residue Avoidance Group, and the Antibiotics Residue Avoidance Group. The CRPs for the compounds to be analyzed are provided to the NRPSSP's Surveillance Advisory Team (SAT) comprised of a group of experts from USDA/FSIS, Food and Drug Administration, Environmental Protection Agency, and Centers for Disease Control and Prevention. The SAT uses the CRP a guideline to evaluate the potential risks associated with the presence of chemicals in meat, poultry and egg products in the NRPSSP selection process. The final decision to include or to exclude a chemical compound from the NRSSP is based on the professional judgment provided by the SAT. Representative CRPs will be discussed to demonstrate how these risk considerations are factored into the NRPSSP selection process for monitoring residues in meat, poultry and egg products.

695 CRITICAL FOLATE PROBLEM WITH "LIFETIME" ASPARTAME AND RELATED STUDIES.

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Much public safety concern arose over the sweetener aspartame by a Soffritti *et al.* (2006) paper published in the NIEHS journal Environmental Health Perspectives (*EHP*). In this study Sprague Dawley (SD) rats provided "lifetime" aspartame treatment developed more leukemias, lymphomas, and schwannomas at the time of natural death compared to untreated controls. Public anger followed a second Soffritti *et al.* (2007) *EHP* paper reporting similar diseases plus mammary tumors in SD dams and pups treated with "lifetime" aspartame, but not in control rats. Subsequently, twelve expert scientists and medical professionals and the Executive Director of the Center for Science in the Public Interest called for a new FDA review. These two *EHP* papers have become the basis for opposition to aspartame, in spite of a comprehensive analysis of aspartame safety by Magnuson *et al.* (2007) that included specific criticisms of both *EHP* papers. Something vital is missing from both Soffritti *et al.* papers and from the comments of Magnuson *et al.* (2007). Folate and vitamin B_{12} deficiency can present as leukemia in humans. Moreover, leukemia, lymphoma, and mammary tumors are folate deficiency-linked in both humans and rats. But both Soffritti *et al. EHP* studies ignored these and three other folate issues. First, SD rats are uniquely susceptible to age-related folate deficiency that can produce DNA and protein damage. Second, pregnant dams can impart folate deficiency to their pups. Third, spontaneous conversion of SD rats from low to high leukemic lines has been reported. These folate liabilities are present in both treated and control rats. Should aspartame or its degradation products diminish folate, these liabilities could distort study conclusions. Existing literature reveals that prolonged treatment of SD rats with the aspartame degradation product methanol causes sustained folate depletion at dosages similar to those of Soffritti *et al.* So, without a folate supplemented, aspartame control group, both Soffritti *et al. EHP* studies and all their related "lifetime" studies may cause folate deficiency in susceptible rats and produce disease not evident in folate sufficient rats.

696 DETERMINATION OF MULTI-CLASS, MULTI-RESIDUE SULFUR-CONTAINING ANTIBIOTICS IN ANIMAL TISSUES USING INTEGRATED PULSED AMPEROMETRIC DETECTION FOLLOWING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY.

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Antibiotics are routinely used in both veterinary and human medicine for therapeutic purposes. These are also commonly used as growth promoters for food-producing animals. If proper withdrawal times are not observed before the slaughtering of medicated animals, meat from these animals may be contaminated with residual

amounts of antibiotics. The presence of such residues in meat is of toxicological and regulatory concern, as some of these may be carcinogenic, cause allergic hypersensitivity reactions, and may also pose antibiotic resistance on humans. Hence, the presence of antibiotics in edible tissues is regulated by the Food and Drug Administration (FDA). For these reasons, it is crucial to develop effective methods for trace level detection of these antibiotics in animal tissues.
Sulfur containing compounds such as sulfonamides, penicillins, cephalosporins, and lincomycin are some of the most effective agents against bacterial diseases and infections. Although most commonly detected by UV absorbance detection, UV detectors suffer from lack of sensitivity especially for β-lactams and lincomycin, which do not posses strong chromophores. This work shows that the reversed phase chromatography coupled to integrated pulsed amperometric electrochemical detection (IPAD) can be used for the determination of multi-class, multi-residue sulfur-containing antibiotics. This study also emphasizes the mechanistic studies of sulfonamides at gold electrodes as well as waveform optimization for sulfur-containing antibiotics using cyclic voltammetry. Excellent sensitivity and analytical figures of merit were obtained, with detection limits in the low ppb level. Developing HPLC-IPAD methods for multi-class, multi-residue sulfur-containing antibiotics can not only be used for antibiotic detection in animal tissues but also can be used in environmental and pharmaceutical applications.

697 ASSESSMENT OF THE HEALTH RISKS POSED BY BENZENE IN CERTAIN SOFT DRINKS.

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A recent study by the FDA indicated that several soft drink beverages contained benzene at concentrations that exceeded the EPA drinking water standard of 5 ppb. Because FDA was attempting to cover a broad range of product types, the number of samples of each individual product was limited. As such, the goal of this study was to better characterize the range of possible concentrations in a representative product and to assess potential human health risks associated with ingestion. Crystal Light Sunrise Classic Orange (CLSCO) was selected as it was reported to have some of the highest concentrations of benzene in the FDA testing. A stratified random sampling approach was developed and samples of CLSCO were obtained from retail outlets in the Houston, Texas area. Since the 16 oz CLSCO product had been reformulated after release of the FDA findings, both old and new formulations were obtained, along with samples of the 64 oz. formulation. Analysis of the samples found mean benzene concentrations of 90 ppb and 0.24 ppb in 16 oz. old and new formulation bottles, respectively, and 3.38 ppb in the 64 oz. product. Point estimates of risk and hazard were developed using average body weights and a 95% upper confidence limit on the mean benzene concentration, as well as by assuming exposure to different formulations of CLSCO over their lifetime and varying body weight based on age. A probabilistic risk assessment was also conducted using distributions for benzene concentration and exposure parameters. Estimates of risk and hazard were slightly greater for teens than for adults, though estimates may not be different when uncertainty is considered. The 50th and 95th percentile lifetime cancer risk estimates were 3.7×10^{-6} and 8.0×10^{-6}, respectively and the 50th and 95th percentile hazard indices were 0.19 and 0.42, respectively. In all cases, regardless of the risk estimation technique used, cancer risk and non-cancer hazard associated with ingestion of benzene in CLSCO appear to be below historical risk levels of concern.

698 THE IMPORTANCE OF ENDOTOXIN CONTAMINATION OF COMMERCIAL PROTEIN PREPARATIONS IN THE CELLULAR EFFECTS OF ADVANCED GLYCATION ENDPRODUCTS.

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Advanced glycation endproducts (AGEs) are generally perceived as having adverse health effects. AGEs are formed by non-enzymatic glycation of proteins and amino acids in food and in vivo. Data have shown that the endogenous AGE load can be influenced by the food AGE content. However, formal proof for a causal relationship between food AGEs and disease is still lacking thus severely hampering the risk assessment of dietary AGEs. Many results on the adverse effects of AGEs were obtained in cellular tests employing in vitro glycated proteins. It is known that many commercial protein sources are contaminated with endotoxin that may contribute to or mask the effects of AGEs. We employed various methods to remove endotoxin from protein preparations and tested these in cultures of Beas2b cells for the induction of inflammatory marker genes using quantitative real-time PCR. Our results show that even protein sources labeled "low-endotoxin" show considerable endotoxin contamination measured with the Pyrogen gel clot assay. Proteins of various sources, glycated or not, induced the expression of the inflammatory marker genes IL-6, IL-8 and TNFα. The induction of inflammatory genes by AGEs was shown to be mediated by RAGE resulting in increased free radical generation, NF-κB activation and expression of pro-inflammatory genes. Our results show that the induction of inflammatory genes by glycated proteins could not be blocked by anti-RAGE antibodies, indicating that the induction was not mediated by RAGE. Removal of endotoxin by extraction with Triton X-114 completely abolished the induction of inflammatory genes. These results demonstrate that complete endotoxin removal of commercial proteins is crucial to determine the effects of AGEs. These observations also suggest that much of the published data on cellular AGE effects may have to be interpreted cautiously because the preparations used were likely contaminated with endotoxin. Our results further suggest that AGEs may be of lesser concern than previously assumed.

699 MODE OF EXPOSURE FOR PHOTOTOXIC COUMARINE DERIVATIVES FROM FOOD AND DRUG EXTRACTS.

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The principal pyranocoumarin species methoxypsoralen is phototoxic and has been associated with an increase in skin cancer incidence. Furano- and pyrancoumarine derivatives are present in a variety of plants, especially in citrus fruits; their specific coumarine content varies widely and depends e.g. on plant type, storage conditions or bacterial contaminations. Pure 5-methoxy- and 8-methoxypsoralens are used for psoralene UV-A therapy; for these patients adverse effect and carcinogenicity data have been published. Due to the coumarin content in herbal plant extracts a carcinogenic warning label has been proposed. Using the mode of exposure (MOE) concept we have compared the coumarine exposure from PUVA therapy and ingestion with the uptake as can be expected by regular use of plant extracts (e.g. angelica extracts for upper abdominal discomfort). PUVA treatment of average duration results in exposure to 12 mg/kg body weight 8-methoxypsoralen. Food basket survey data transfer into a lifetime uptake of 5.1 mg/kg bw psoralene (assuming 1% psoralene content). From intake of gerbal extracts for a long ailment of 10 years with three treatment episodes per year the additional lifetime uptake of coumarines is 0.027 mg/kg bw (coumarine content comparable to commericial extracts from Archangelica species). From these data the MOE for coumarines from herbal extracts is 0.007, i.e. 0.7% of the coumarines present in food; even continuing use for 10 years increases the MOE only to 0.03. These calculations include conservative assumptions for psoralene content in coumarine mixtures and phototoxic potential of coumarines other than psoralenes; they result in an overestimation. The additional skin cancer risk from herbal drug coumarine content is low both to total nutrient content and interindividual variation and does not warrant a warning label for herbal extracts, but not nutrients.

700 INDIVIDUAL AND COMBINED EFFECTS OF OCHRATOXIN A AND FUMONISIN B1.

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Multi-contamination of foodstuffs by mycotoxins occurs. Among combinations those of ochratoxins (OTA) produced by Aspergillus and Penicillium species and fumonisins (FB1) by Fusarium species are frequent. They are increasingly implicated in diseases causation animals and humans. Their modes of action in cytotoxicity and carcinogenesis have been studied individually these last decades however controversies remain. The genotoxicity of ochratoxin A is mostly admitted whereas that of fumonisin B1 remains questionable. Since they may operate through both genotoxic and epigenetic mechanisms, we have studied the mechanisms of their toxic effects when combined. To set up a more comprehensive mechanistic scheme of their carcinogenic process either individually or combined, some of their epigenetic effects have been evaluated in different cell lines, Vero cells, C6 rat glioma cells, human Caco-2 cells and p53-null MEF mouse cells. Using the 3D test (DNA repair test, NERS and BERS) we confirmed that OTA induced DNA damages requiring repair systems whereas FB1 did not. The results also showed that OTA and FB1 operate in the different cell lines through some non genotoxic mechanisms, oxidative stress, indirect hyper methylation or oxidation of DNA (m5dC) and/or 8OHdG, inhibition of gap junction intercellular communication (GJIC). All these

effects are known to interfere with cell growth and DNA replication. We conclude that (i) OTA may display genotoxic effects and epigenetic ones, both leading jointly to carcinogenesis; (ii) they may be combined or synergistic in carcinogenesis process as well as in other organ toxicities in the presence of FB1; (iii) at present epigenetic effects of mycotoxins are most likely under evaluated versus direct genotoxic effects; (iv) The risk assessment for humans potentially exposed to combinations of mycotoxins should be revised.

701 VALIDATION OF URINARY FREE FUMONISIN B1 AS A BIOMARKER OF EXPOSURE.

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Fumonisin B1 (FB1) is a carcinogen and a tumor promoter in animal studies and was categorized as possibly carcinogen to humans (Group 2B) by IARC. Associations between dietary FB1 exposure and neural tube defect or esophageal and liver cancers have been suggested by studies in the US, South Africa, and China. To assess the potential human health risks, development and validation of specific FB1 biomarkers is urgently needed. The correlation of urinary free FB1 with FB1 exposure in animal model has been reported. In this study, urinary excretion of FB1 was evaluated as a potential biomarker for human dietary exposure to FB1 in two populations in China. Morning urine samples were collected from 76 healthy participants, 43 from Huaian, a high-risk area of esophageal cancer, and 33 from Fusui, a high-risk area of liver cancer. Free FB1 in urine was determined by immunoaffinity column-HPLC-fluorescence detection, and adjusted by urinary creatinine level. In addition, 148 food samples collected from these two areas were analyzed for FB1. Free FB1 was detectable in 83.7% (36/43) Huaian urine samples and 84.8% (28/33) Fusui urine samples, which was no significant difference ($p>0.05$). However, the mean urinary free FB1 level in Huaian subjects, 13.62 ± 42.72 (range 0.06-253.61) ng/mg creatinine, was significantly higher than that in Fusui subjects ($p<0.001$), which was 0.72 ± 0.85 (range 0.01-37.2) ng/mg creatinine. Based on consumption record and measured FB1 levels in food, daily FB1 intakes were estimated. About 95.3% (41/43) Huaian subjects had their daily FB1 intakes greater than 120 μg (JECFA tolerable total daily intakes of FB1/60 kg body weight), which is significantly higher than 64.7% (21/33) in Fusui subjects ($p<0.05$). These data demonstrated that significant difference of urinary free FB1 levels in the two populations reflects significant difference of dietary FB1 intakes, which provides supportive evidence for the use of urinary free FB1 as a biomarker of exposure (supported by NIH grants CA94683 and CA90997).

702 NEUROTOXIC EFFECTS OF MANGANESE ON THE DOPAMINERGIC INNERVATION OF THE GILL OF THE BIVALVE MOLLUSC, *CRASSOSTREA VIRGINICA.*

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Manganese casuses Manganism, a Parkinson-like disease, in individuals exposed to high airborne levels. Symptoms include include gait imbalance, rigidity, tremors, bradykinesia and dystonia, which occurs because manganese injures dopaminergic neurons involved in control of body movements. Lateral cilia of gill of *Crassostrea virginica* are controlled by a serotonergic-dopaminergic innervation from their ganglia. The nervous system of the animal is simple with monoaminergic neurons and measurable amounts of biogenic amines. Serotonin is an excitatory neurotransmitter causing cilio-excitation. Dopamine acts as an excitatory neurotransmitter within the ganglia, an inhibitory neurotransmitter at the gill, causing cilio-inhibition. We examined effects of manganese treatments on the nervous system and innervation of the gill of *C. virginica*. Animals were treated for 3 days with manganese. Beating rates of the lateral cilia were measured in gill preparations which had the ipsilateral cerebral and visceral ganglia attached (CG preparations), only the ipsilateral visceral ganglia attached (VG preparations) and isolated gill preparations. Manganese treatments resulted in significant impairments of the dopaminergic system while having no effect on the serotonergic system. Amine levels in ganglia and gill were measured by HPLC with fluorescence detection. The treatments caused significant reductions in dopamine in the cerebral ganglia, visceral ganglia and gill, but not serotonin. We also tested PAS (p-aminosalicylic acid) and found it protected the ganglia and gill against the effects of 0.5 mM manganese, but not 1 mM manganese. The study shows this preparation is a useful model to study manganese neurotoxicity, the pharmacology of drugs affecting it and biogenic amines, and the findings may provide insights into the actions of manganese and PAS in treatments of Manganism. This work was supported by 2R25GM0600305 of NIGMS, 0516041071 of NYS-DOE, 0622197 of NSF and 678760036 of PSC-CUNY.

703 POTENTIATION OF PROINFLAMMATORY CYTOKINES BY MANGANESE IN LPS-ACTIVATED MICROGLIA IS ASSOCIATED WITH DECREASED EXPRESSION AND ACTIVITY OF MITOGEN ASSOCIATED PROTEIN KINASE PHOSPHATASE-1.

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In previous work we have demonstrated that manganese (Mn) exposure potentiates inflammogen (lipopolysaccharide; LPS)-induced proinflammatory cytokines (e.g. IL-6 and TNF-α) production by N9 microglial cells ant that this potentiation is associated with enhanced p38 mitogen activated protein kinase alpha (p38α) activity. To explain the observed enhanced p38α activity, we hypothesized Mn either augmented the activity of the kinase upstream of p38α (MKK-3/6) or decreased activity of the phosphatase down-stream of p38α (mitogen activated protein kinase phosphatase-1; MKP-1). Using western blot analysis, we examined MKK-3/6 activity following exposure to Mn (250 μM), LPS (100 ng/ml), or Mn-LPS for 15min, 1h, or 4h. We observed that Mn in combination with LPS increased phosphorylation of MKK-3/6 at all time points. However, the observed activation was not significantly different from the increased phosphorylation caused by LPS alone; suggesting the enhanced p38α activity in the presence of Mn was not due to increased MKK-3/6 activation. Next, we examined the downstream phosphatase, MKP-1, by western blot and quantitative PCR. One-hour exposure to Mn alone or in combination with LPS decreased the protein expression of MKP-1 relative to the MKP-1 levels in control and LPS-exposed cells, respectively. In agreement with the western blot data, similar decreases in MKP-1 mRNA at 1h after exposure were observed in Mn-exposed cells regardless of the presence of LPS. Thus, it appears that, at least in part, the enhanced p38α activity and proinflammatory cytokine production caused by Mn are due to decreased MKP-1. To determine the temporal relationship between the decreased MKP-1 expression observed in this study and the enhanced cytokine production we have observed previously, in future studies we will examine the time-course of the expression of proinflammatory cytokine and MKP-1 mRNA levels. (Supported by NIEHS ES11654).

704 NF-κB-DEPENDENT EXPRESSION OF NITRIC OXIDE SYNTHASE 2 IN ASTROCYTES IS POTENTIATED BY MANGANESE *VIA* ACTIVATION OF SOLUBLE GUANYLATE CYCLASE AND EXTRACELLULAR REGULATED KINASE SIGNALING PATHWAYS.

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Inflammatory activation of glial cells is associated with neuronal injury in several degenerative movement disorders of the basal ganglia, including manganese (Mn) neurotoxicity. Overexposure to Mn can produce neurotoxic levels of inflammatory mediators such as nitric oxide (NO) and pro-inflammatory cytokines, including interferon-gamma and tumor necrosis factor-alpha (I/T). It has been hypothesized in the present studies that upstream signaling events involving MAP kinases and soluble guanylyl cyclase (sGC) pathways underlie the capacity of Mn to enhance cytokine-dependent activation of NF-kappa B (NF-κB). Coexposure of primary astrocytes to 10 μM Mn and I/T significantly potentiated both steady state levels of nitric oxide synthase 2 (NOS2) mRNA, protein and production of NO when compared to Mn and I/T alone. In order to determine NF-κB's role, a dominant negative mutant of IκBα was used and significantly decreased NOS2 mRNA, protein and NO production, which was normally induced by co-exposure of Mn and cytokines. Activation of ERK, p38 and JNK rapidly increased upon exposure to Mn and I/T, as well as an increase in NF-κB promoter activity. Upon inhibition of ERK activation a decrease in NF-κB activity and NO production is seen indicating ERK precedes NF-κB in astrocytes exposed to Mn and I/T. Independent of cytokines, Mn increases cGMP synthesis and sGC inhibition decreased ERK and NF-κB activity as well as synthesis of NO. These data indicate that near physiologic concentrations of Mn potentiate cytokine-induced expression of NOS2 and production of NO in astrocytes via activation of sGC that promotes ERK-dependent enhancement of NF-κB signaling.

705 MANGANESE PRIMARILY ACCUMULATES IN NUCLEUS OF CULTURED BRAIN CELLS.

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Manganese (Mn) is known to pass across the blood-brain barriers and interact with dopaminergic neurons. However, the knowledge on the subcellular distribution of Mn in these cell types upon exposure to Mn remained obscure. This study was designed to investigate the subcellular distribution of Mn in blood-brain barrier endothelial RBE4 cells, blood-cerebrospinal fluid barrier choroidal epithelial Z310

cells, mesencephalic dopaminergic neuronal N27 cells, and pheochromocytoma dopaminergic PC12 cells. The cells were incubated with 100 μM MnCl2 with radioactive tracer 54Mn in the culture media for 24 hrs. The subcellular organelles, i.e., nuclei, mitochondria, microsomes, and cytoplasm, were isolated by normal or ultra-centrifugation and verified for their authenticity by determining the markers specific to cellular organelles. Data indicated that the maximum Mn accumulation was observed in PC12 cells, which was 2.8, 5.2 and 5.9 fold higher than that in N27, Z310 and RBE4 cells, respectively. Within cells, about 92%, 72%, and 52% of intracellular 54Mn were found to be present in nuclei of RBE4, Z310, and N27 cells, respectively. 54Mn in PC12 cells was recovered by 27% from nuclei and 69% from cytoplasm. Surprisingly, less than 0.5% and 2.5% of cellular 54Mn was found in mitochondrial and microsomal fractions, respectively. This study suggests that that nuclei may serve as the primary target for Mn cytotoxicity; mitochondria and microsomes may play an insignificant role in Mn subcellular distribution.

706 DEVELOPMENTAL VULNERABILITY TO MANGANESE-INDUCED BEHAVIORAL DYSFUNCTION.

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Chronic overexposure to manganese (Mn) results in Manganism, a neurodegenerative disorder of the basal ganglia. Adult susceptibility to manganese is well-studied, but the relevance of exposure during development to vulnerability later in life is poorly understood. We therefore postulated that mice exposed to Mn during development would exhibit a greater degree of neurologic dysfunction upon secondary exposure as adults than mice that were only exposed as adults. 20-day old C57BL/6 mice were weighed and received gastric gavage daily, for two weeks, of weight-adjusted doses of either saline, 10 mg/kg MnCl2, or 30 mg/kg MnCl2. At age 12 weeks the same mice were again exposed to the same regime of weighing and daily gavage of weight-adjusted doses of MnCl2, this time for an eight week period. Also, a group of 12 week old mice that had not been exposed as juveniles went through the same protocol as that of the re-exposed adults. Neurologic function was determined prior to exposure and throughout both exposure periods using a non-invasive activity monitoring system that uses infrared technology to track motion in xyz planes. Preliminary analysis of indicators for neuro-behavioral function indicate that the variables total movement, rearing movement, and time spent moving are different in the doubly exposed mice than in the adult-only exposed mice. Additionally a difference in dysfunction between genders was noted with greater damage in males than females to the extent that high dosage had to be changed from 100mg/kg to 30mg/kg because 100mg/kg dosage resulted in 75% male death. This supports the hypothesis that juvenile exposure to Mn enhances vulnerability in mice re-exposed as adults and identifies a gender difference in sensitivity to Mn in developing animals.

707 EFFECTS OF CHRONIC MANGANESE EXPOSURE ON GLUTAMATERGIC MARKERS IN THE NON-HUMAN PRIMATE BRAIN.

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Manganese (Mn) is an essential element that is required for normal human physiological processes. Environmental, occupational, and iatrogenic exposures, as well as certain medical conditions result in excessive accumulation of Mn in the brain. The neurological sequelae of exposure to toxic levels of Mn include psychiatric, cognitive and motor deficits, suggesting the involvement of multiple brain regions and neuronal systems. Emerging evidence suggests that Mn causes dysregulation of glutamatergic synapses. We have recently shown that Mn inhibits the NMDA receptor channel in neuronal membrane preparations from rat brain (Guilarte and Chen. Neurotoxicol., 2007). In the present study, we used quantitative autoradiography of NMDA receptors and HPLC analysis of glutamate and glycine levels in different brain regions of the non-human primate brain. *Cynomologous macaques* were given injections of 3.3-5.0, 5.0-6.67 or 8.33-10 mg Mn/kg for several months. Glutamate and glycine levels were detected by HPLC in 13 different brain areas in Mn-treated and control monkeys. Preliminary analysis indicates that glutamate levels are significantly increased (44%; $p<0.05$) in the visual cortex of animals treated with the highest Mn dose. However, glycine levels were decreased (30%) in the deep cerebellar nuclei in these same animals. [^{3}H]-MK801 specific binding to NMDA receptors was used to visualize and measure their distribution throughout the cerebral cortex, basal ganglia, hippocampus, and cerebellum. NMDA receptor levels were unchanged in 22 of 23 brain areas measured, with a statistically significant decrease (22%) only in area 9/46D of the frontal cortex in animals with the highest Mn dose. Glutamate transporter distribution throughout the brain is also being examined by quantitative autoradiography. This study seeks to further describe possible mechanisms by which Mn disrupts neurological function. [Supported by NIEHS grant ES010975 to TRG]

708 MANGANESE EXPOSURE IN 328 SMELTING WORKERS: RELATIONSHIP AMONG EXTERNAL/ INTERNAL MARKERS AND NEUROLOGICAL/PSYCHOMOTOR EXAMINATIONS.

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Reliable biomarkers for Mn exposure are not available at present. The purpose of this cross-sectional study was to explore the relationship between airborne Mn levels (MnAir) and changes in biological and neurological parameters among smelters who are exposed to Mn in manufacturing ferroalloys. Chinese workers representing control, low and high exposures were recruited with each group consisting of 110, 104, and 114 subjects, respectively. The airborne Mn levels were <0.04 mg/m3, 0.04-0.1 mg/m3 and >0.1 mg/m3 for control, low and high exposure groups, respectively. Mn levels in serum, red blood cells (MnRBC), saliva (MnSa) and hair were significantly higher in Mn-exposed subjects than controls. Linear regression revealed that MnRBC and MnSa were significantly associated with MnAir ($r=0.71$, $p=0.02$ for MnRBC; $r=0.58$, $p=0.025$ for MnSa). Interestingly, when the ratio of Mn/Fe in RBC from each subject was used for analysis, the mean Mn/Fe ratios (0.47±SD0.46, 0.93±0.39 and 2.02±1.26 in control, low and high exposure groups, respectively) represented a respective 98% and 345% increase in Mn-exposed subjects, percentages that are much greater than those using MnRBC or MnSa alone. The Mn/Fe ratio in RBC was also significantly correlated with the inhalable MnAir ($r = 0.56$, $p<0.01$). Additionally, analysis of movement dysfunction using the Purdue Pegboard, indicated that highly exposed workers ≥ 40 years of age produced scores that were significantly lower than 1) control subjects who were in the same age group or 2) high Mn exposed smelters < 40 years of age. Taken together, our data suggest that smelting workers are at risk when exposed to airborne Mn. The ratio of Mn/Fe in RBC may be a better estimate for Mn exposure assessment. Support in part by NIEHS ES 08164 and DoD USAMRMC W81XWH-05-1-0239

709 HEPATOTOXIC REACTION ASSOCIATED WITH A PRODUCT CONTAINING "BLACK COHOSH": ONE CASE OF MISTAKEN IDENTITY.

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Sponsor: G. Bondy.

The use of black cohosh (BC, *Cimicifuga racemosa*, syn. *Actea racemosa* L.) has gained popularity for the relief of menopausal symptoms. In recent years, over 50 international cases of hepatotoxicity (including liver transplants) suspected as being associated with the use of BC, have prompted reviews of this herb's safety. Adverse reaction reports submitted to Health Canada (HC) were reviewed. Four serious cases of hepatotoxicity associated with the use of BC were reported to HC as of June 2007. The causality was assessed as "possible" in three cases (due to confounding concomitant medications and other medical conditions) and as "probable" in one case, according to WHO causality assessment criteria. The probable case of hepatitis had no identifiable confounders, and was associated with a time to onset of 9 days following daily use of a product labelled as containing 100 mg BC root extract/powder. Samples of this product were analyzed for phytochemical markers to confirm the authenticity of the BC. Analysis was conducted by LC/MS using U.S. Pharmacopeia BC powder as a standard. The results were positive for actein and 23-epi-26-deoxyactein (common to *Actea* species, including authentic BC) and cimifugin (a marker for Asian *Actea* species) but negative for cimiracemoside-C (a marker for authentic BC). Other studies have shown that some commercially available BC products contain Asian *Actea* species rather than authentic *C. racemosa*, and this study links a probable case of hepatotoxicity with such a product. While certain *Actea* species are toxic, the possible inherent toxicity of *C. racemosa* needs to be further researched. This study shows that some cases of hepatotoxicity associated with products purported to contain BC may be unrelated to authentic *C. racemosa*. Plant species identification is an important factor in accurately assessing the association between products containing BC, and hepatotoxicity.

710 USNIC ACID-INDUCED CHANGES IN THE TRANSCRIPTIONAL PROFILE OF MITOCHONDRIAL GENES IN MOUSE LIVER.

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The use of dietary supplements containing usnic acid for weight loss has been reported to be associated with hepatotoxicity in humans, particularly in women. Usnic acid is a lichen metabolite that exerts its action by uncoupling mitochondrial oxidative phosphorylation. Several in vitro studies have been conducted to evaluate the uncoupling effect of usnic acid on mitochondrial function, primarily by determining the rate of oxygen consumption. Also, information regarding toxicity of usnic acid in animals is limited. Here, we report the molecular mechanism of usnic acid-mediated changes in liver mitochondria of female B6C3F1 mice. Using a mitochondria-specific oligonucleotide microarray consisting of 542 genes associated with mitochondrial structure and functions, we examined the effect of usnic acid on the mitochondria. Beginning at 8 weeks of age, animals received usnic acid at 0, 60, 180, and 600 ppm in NIH-31 diet for 14 days. Microarray analysis showed the change in expression level of mitochondrial genes only at 600 ppm, the highest dose of usnic acid. A significant increase in expression level of genes associated with complex I, II, III, and IV was observed in response to 600 ppm while complex V was unaltered. In addition, several genes associated with fatty acid oxidation pathway, TCA cycle, and apoptosis were up-regulated at the highest dose. These results suggest increased electron transport activity of respiratory chain complexes. However, fatty acid oxidation pathway may have been activated to generate ATP due to uncoupling of oxidative phosphorylation in response to 600 ppm of usnic acid in the liver of female mice.

711 CARDIOPROTECTIVE ACTIVITY OF RESVERATROL IN RATS.

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Resveratrol (trans-3,5,4'-trihydroxystilbene) is a phytoalexin that is present in several natural products, notably the skin of red grapes. On the basis of its anticarcinogenic activity in *in vivo* models, resveratrol is being developed as a cancer chemopreventive agent. As part of this development, the subchronic oral toxicity of resveratrol was evaluated in rats. Groups of 20 CD rats per sex received daily oral (gavage) exposure to resveratrol at doses of 0, 200, 400, or 1000 mg/kg/day for 13 weeks. Toxicology endpoints included survival, clinical observation, body weight, food consumption, clinical chemistry, hematology, coagulation, ophthalmology, neurotoxicity (via functional observational battery), organ weights, and gross and microscopic pathology. Administration of resveratrol induced no mortality or clinical evidence of toxicity in any study animal. Resveratrol had no effect on body weight in male rats; however, statistically significant, dose-related reductions in body weight gain were seen in female rats in the middle and high dose groups. Total bilirubin was increased in both sexes in the high dose group; other clinical pathology parameters were within normal limits in all groups, as were the results of ophthalmology and neurotoxicity evaluations. Organ weights were comparable in all groups. Gross pathology was unremarkable, and no significant microscopic lesions were seen in any rat treated with resveratrol. Interestingly, the incidence and severity of cardiomyopathy in male and female rats exposed to the high dose of resveratrol were significantly reduced in comparison to rats in the vehicle control group. These data suggest that the chemoprotective activity of resveratrol may not be limited to neoplastic development, as subchronic administration of this agent also appears to protect against cardiomyopathy. The No Observed Adverse Effect Level for subchronic oral administration of resveratrol to rats is 200 mg/kg/day. (N01-CN-43304)

712 CYTOTOXICITY SCREENING OF AYURVEDIC HERBAL EXTRACTS USING *IN VITRO* TECHNIQUES AND SCANNING ELECTRON MICROSCOPY.

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Diabetes mellitus, a physiologic disorder characterized by predisposition to developing hyperglycemia causing tissue damage, has become a disease of worldwide significance. Recently, patients have utilized alternative medicine for treatment including herbs of the Indian therapy system Ayurveda, an ancient medical science. Herbs are taken orally by ingestion of the aqueous extract. Research has shown that various Ayruvedic herbs promote hypoglycemic activity in vivo; however, there is insufficient data concerning their safety and efficacy. The effects of four Ayurvedic herbs, and one non-Ayurvedic cytotoxic herb, on the LLC-PK1 porcine normal renal epithelial cell line were investigated. Cell viability was determined by the trypan blue exclusion assay. The LC50 values for the Ayurvedic herbs and non-Ayurvedic herb are as follows (in mg/mL): Aegle marmelos (Bael Leaf), 0.65 ± 0.16; Andrographis paniculata (Kalmegh), 0.14 ± 0.06; Hemidesmus indicus (Anantmool), 0.98 ± 0.36; Wrightia tinctoria (Indrajav), 0.84 ± 0.12; Scutellaria lateriflora (Skullcap), 0.45 ± 0.07 (non-Ayurvedic positive control). Results indicate Kalmegh to be the most cytotoxic herb of those assayed. Phase-contrast light microscopy was used to observe changes in cellular morphology, which were seen in all herbal extracts after a twenty-four hour treatment.
Scanning electron microscopy with x-ray microanalysis was used to qualitatively detect lead and mercury in crude herbal samples due to studies suggesting select crude Ayurvedic herbs to be contaminated with traces of these toxic metals. Results show no traces of lead or mercury in all crude samples assayed. Based on results of the trypan blue assay and phase-contrast light microscopy, further investigation of the toxicology of Ayurvedic herbs is necessary.

713 CYTOTOXICITY OF STILBENE COMPOUNDS IN TRANSFORMED CELLS.

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Stilbenoids are natural plant products that exhibit diverse biological activities on mammalian cells. The toxicity potential of these compounds remains to be fully defined. Using a MTT-based assay, the present study has evaluated the *in vitro* toxicity of five stilbene analogs towards transformed mouse macrophages (RAW264.7). While trans-stilbene and trans-stilbene oxide exhibited relatively low toxicity (LC50 > 100 and >200 μM, respectively), resveratrol (RES), piceatannol (PIC) and trans-pterostilbene (TPS) demonstrated greater toxicity (LC50 29.1, 1.3 and 8.3 μM, respectively). In contrast to unstimulated macrophages, stimulation with 0.5 ng/mL lipopolysaccharide (LPS) conferred significant protection against RES toxicity, as determined by MTT assay and scanning electron microscopy. The antiproliferative capacity of RES and PIC was evaluated in three transformed cell types (RAW264.7 and 10ScNCr/23 mouse macrophages, CEM human T lymphoblasts, and human A431 skin cells). Paradoxically, while higher concentrations of RES (25 and 50 μM) inhibited the proliferation of all cell types, a low concentration (5 μM) stimulated the proliferation of RAW264.7 macrophages. In contrast, the antiproliferative effects of PIC, a metabolite of RES, varied in each cell type. In particular, macrophages deficient in Toll-like receptor 4 (TLR4) were completely resistant to the growth inhibitory effects of PIC. Taken together, these results indicate that despite similarities in stilbenoid chemical structure, the viability of RAW macrophages is most affected by hydroxylated stilbenoids such as RES, PIC and TPS. Toxicity of these compounds on LPS-stimulated macrophages was significantly less than on unstimulated macrophages. The effects of these compounds on cell proliferation varied according to concentration and cell type, however, the lack of growth inhibitory effects of PIC treated macrophages deficient in TLR4 suggests that these receptors may play a role in modulating cell proliferation.

714 OXYMATRINE REDUCES DMSO-INDUCED TOXICITY IN H4IIE CELLS.

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Toxic chemicals can induce stress and liver injury. The protection of cells from chemical toxicity is important in reducing liver damage. The management of stress from dimethyl sulfoxide (DMSO) in liver cell lines is studied. Oxymatrine, one of the major alkaloids in Sophora japonica also known as KuShen, is known to have health benefits on the liver. However, the biologic activity of oxymatrine remains unclear. The present study aims to study effects of oxymatrine on H4IIE after treatment of the cell line with DMSO. H4IIE cell line was incubated with oxymatirine and DMSO for different periods of time. The cell viability was measured by Neutral Red (NR) assay. Flow cytometry was performed to study the influence of oxymatrine on the cell differentiation. The results showed for cells without oxymatrine pre-treatment, cell injury was implicated as indicated by the decrease in cell viability. The time-course study of oxymatrine showed pretreatment of cells for 24 h was effective. The LC50 of oxymatrine in H4IIE was <1.5 mM. Oxymatrine showed protective effects on cells from the DMSO-induced toxicity. Cytometry indicated that oxymatrine can inhibit the G2 and M phase of H4IIE at 1.5mM. The findings suggest that oxymatrine could inhibit cell division of cancer cell H4IIE, and could protect H4IIE cell line from DMSO-induced oxidative stress due probably to its capacity to inhibit the metabolic activation of hepato-toxin, a critical factor in the pathogenesis of chemical-induced liver injury.

715 SUBCHRONIC SAFETY EVALUATION OF A UNIQUE COMPOSION OF DEFIND EXTRACTS OF SCUTELLARIA BAICALENSIS AND ACACIA CATECHU.

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A standardized plant composition - UP446 with primarily baicalin from the roots of Scutellaria baicalensis (Ref. US07192611) and (+)-catechin from the heartwoods of Acacia catechu (Ref. US07108868) has been used in both joint supplements and a prescription medical food. This natural flavonoid composition reduces production of eicosanoids through dual inhibition of COX and LOX enzymes and also decreases gene and protein expressions of pro-inflammatory cytokines, IL-1beta, IL-6, and TNF-alpha (Ref. Burnett et al. J. Medicinal Food, 10(3), 442-451, 2007). The in vitro and in vivo safety evaluations of UP446 have been reported (Ref. Burnett et al J. Food Biochem. In press, 142, 2007).
A supplement 90-day oral toxicity study was conducted in Hsd:SD rats to determine the potential of UP446 to produce toxicity. Four groups of animals (10 males and 10 females per group) with daily oral administration of UP446 at dose levels of 250, 500 and 1,000 mg/kg/day against a vehicle control (0.5% solution of carboxymethylcellulose in distilled water) were tested. Animals were observed for ophthalmic changes, signs of gross toxicity, behavioral changes, body weights, feed consumption, sperm count for males and estrus cycle staging for females. Blood was sampled for hematology, clinical chemistry and coagulation assessments. Urine was collected before necropsy. Gross necropsies were performed on all rats, and over 43 organs and tissues from the 1,000 mg/kg/day and control groups were evaluated histologically.
There were no test article related mortalities or ophthalmological, neurological (functional observational battery and motor activity), body weight gain, food consumption, clinical observation, organ weight, gross finding, clinical or histopathologic alterations. Normal sperm count and comparable estrus staging were observed.
Daily administration of UP446 by oral gavage at dose levels of 0, 250, 500 and 1,000 mg/kg/day for 90 days produced no evidence of toxicity. A dose of 1,000 mg/kg/day was identified as the NOAEL (no-observed-adverse-effect-level) in this study.

716 TOXICITY EVALUATION OF PHYLLANTHUS AMARUS SCHUM &THONN EXTRACTS.

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Phyllanthus amarus is an herbal medicinal drug used to treat mainly diabetes. The purpose of this study is to evaluate its toxicity. The cytotoxicity of aqueous extracts (AE) and hydro alcoholic extracts (HAE) of Phyllanthus amarus on human adenocarcinoma cells Caco-2 was evaluated using neutral red uptake and MTT test. Aqueous extract was more cytotoxic than hydro alcoholic extract (IC50 89.6 µg/ml versus 277 µg/ml respectively for AE and HAE). The acute and sub chronic toxicity of the same extracts were evaluated in Swiss mice and Wistar rats respectively. A single oral dose of 5g/kg of each extract did not produce mortality or any significant change in the general behaviour of the treated mice compared to control ones over a 14-day observation period. Extracts were administrated daily by oral route at 1 and 3g/kg for 28 days to rats. There were no significant differences in body weight gain and blood glucose levels in treated rats as compared to controls. Clinical biochemistry did not reveal any pathological modification. Neither gross abnormalities nor histopathological changes in liver, kidney and pancreas were observed.
P. amarus extracts could be classified as non toxic (LD 50% > 5g/kg mouse and >3g/kg rat) according to international regulations, however it is slightly cytotoxic to the human adenocarcinoma cell line Caco-2.

717 THE EFFECTS OF CHRONIC ALOE VERA ADMINISTRATION ON COLONIC PROTEIN EXPRESSION AND GLOBAL DNA METHYLATION IN THE FISHER-344 RAT.

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Aloe vera, traditionally used as a topical cosmetic ingredient and a dietary supplement, is widely promoted as a prophylaxis in the treatment of a variety illnesses and gastro-intestinal disorders. The Aloe vera latex contains C-glycoside anthraquinones that are unique to the Aloe species and are responsible for the cathartic effects of Aloes in humans and animals. In a previous 90-day dosed water study on Aloe vera, we found decreased transit times and reduced body weights in rats. Gross pathology changes related to Aloe vera administration were limited to the cecum and large intestine, where hyperproliferation of goblet cells were observed. In the present study, we administered the lyophilized powder of Aloe vera whole leaf extracts to rats in the drinking water at concentrations of 0.0, 0.5, 1.0, and 1.5% (wt/wt) for two years to determine the long term effects of Aloe vera on colonic protein expression and global DNA methylation. Ingestion of Aloe vera was associated with increased fluid intake and decreased body weight gains. Western blot analyses showed that Cox -1 was expressed at a constant level in rat colon, while Cox-2 was differentially expressed, especially in the colon of rats administered the 1.5% Aloe whole leaf extract. Significant dose-related DNA hypomethylation was observed in the colons of rats administered Aloe vera, and pairwise comparisons with untreated controls were significant at all Aloe vera concentrations. Since alterations in DNA methylation patterns have been associated with genomic instability, our results suggest a potential mechanism for Aloe vera toxicity. Research supported by IAG 224-93-0001 between the FDA and the NTP.

718 PURIFICATION AND TOXICITY CHARACTERIZATION OF SATRATOXIN G AND RORIDIN L2 FROM THE BLACK MOLD STACHYBOTRYS CHARTARUM.

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Stachybotrys chartarum, the black mold which has been associated with damp building-related illnesses, produces satratoxin G (SG), a macrocyclic trichothecene that induces neuronal cell apoptosis in vitro and nasal olfactory sensory neuronal (OSN) death, rhinitis and focal encephalitis in the mouse nose. A major impediment to SG toxicity studies is that this toxin is not commercially available and methods for its production/isolation are poorly described. The purpose of this research was to develop an optimized method for production and purification of SG from S. chartarum cultures and verify its biologic activity. Stachybotrys chartarum 29-58-17 was cultured on autoclaved rice (5x105 spores /250g rice/Fernbach flask) for 4 wk and extracted with acetonitrile. The extract was evaporated to dryness, redissolved in dichloromethane, filtered and concentrated further. Approximately 5 g of the slurry extract was subjected to Michel-Miller silica gel chromatography using a step-wise acetonitrile-dichloromethane gradient system. HPLC and mass spectroscopy data showed that SG was eluted in acetonitrile-dichloromethane (30+70) fraction whereas roridin L2 (RL2), second trichothecene that co-chromatographed with SG on HPLC, was eluted in acetonitrile-dichloromethane (40+60) fraction. The ratio of SG to RL2 was 3:1 in the crude extract. SG and RL2 fractions were pooled, evaporated and subjected to repeated C18 HPLC chromatography using an acetonitrile-water gradient for final purification. SG (10-25 ng/ml) and (100 µg/kg bw) induced apoptotic death in the PC-12 neuronal cell line and of OSN in mouse olfactory epithelium, respectively, while RL2 at equivalent doses had no effect in these assays.

719 ACUTE HEPATOTOXICITY OF USNIC ACID IN FISHER 344 RATS AND B6C3F1 MICE.

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Usnic acid, a secondary metabolite of Usnea sp., and an active ingredient in many dietary supplements; some of which are directly associated with severe liver problems observed among consumers. To establish appropriate dose of Usnea lichen and pure usnic acid, we conducted range-finding studies in Fisher 344 and B6C3F1 mice. At higher doses of 2500 and 1250ppm for rats and 1200 and 600ppm for mice, usnic acid caused severe weight loss and early death. No significant reduction in weight was observed in animals of lower dose groups (60 -350 ppm). Studies showed that the lichen which was added to the feed to achieve equivalent usnic acid concentrations, showed more toxicity than pure usnic acid. At the highest dose, both mice and rats died within three days. Livers were evaluated for hepatotoxicity in all the studies. Usnic acid was seen to cause hepatotoxicity, apoptosis and inflammation. Hepatocellular apoptosis around the portal vascular system was observed by staining active caspase-3 in paraffin sections of liver. Usnic acid induced cytochrome P450s in rat liver. At the higher dose groups, hepatic pentoxyresorufin O-dealkylase activity was increased 9.5-fold in female and 6-fold in male rats, while hepatic testosterone 6beta- hydroxylase was induced 8-fold in females and 3-fold in males. These inductions were associated with increased CYP2B1 and CYP3A2 expression, confirmed by western blotting. In summary, though high concentrations of usnic acid shows significant weight loss, its therapeutic value is questionable due to the observed hepatotoxicity. Research was supported in part by IAG 224-93-001 between the NCTR/FDA and NIEHS/NTP.

720 SUBCHRONIC TOXICITY OF PULEGONE ADMINISTERED BY GAVAGE TO FISCHER344 RATS.

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Pulegone, a naturally occurring constituent of plants in the mint family, is used as a flavoring agent and in herbal products. Because of the wide use of pulegone and the limited known health effects, these studies were performed to provide data for subsequent chronic studies. Male and female Fischer 344/N rats (10/sex/group) were administered pulegone (~96% pure) by gavage in corn oil at dosages of 0 (control), 9.375, 18.75, 37.5, 75, or 150 mg/kg, 5 days/week, for approximately 13 weeks. Only one rat (150 mg/kg female) died during the study. Group mean body weights were similar to controls at study termination for all treated groups except the 150 mg/kg males (57% of control), 150 mg/kg females (78%), and 75 mg/kg male (87%) rats. Treatment related increases in liver, thymus, and kidney weights (absolute and/or relative) were noted. At termination, significant increases in segmented neutrophils, lymphocytes, total white cells, platelets, bile salts/acids, gamma glutamyltranspeptidase, alkaline phosphatase, and alanine aminotransferase activity were observed while a significant decrease in red blood cells, hemoglobin, and hematocrit were described in pulegone treated rats. Also, an increase of reduced and/or oxidized glutathione concentration in the liver of the pulegone treated rats was present at termination. Treatment-related microscopic findings in the liver (both sexes) included hepatocellular hypertrophy, periportal fibrosis, oval cell hyperplasia, bile duct hyperplasia, hepatocellular necrosis (male only) and serosal fibrosis (female only). Other treatment-related findings also included were bone marrow hyperplasia, glandular stomach and heart mineralization, and renal tubular protein casts. (Supported by N01-ES-15460)

721 THE ESSENTIAL OIL OF LIPPIA ALBA PROTECTS AGAINST THE CYTOTOXIC AND GENOTOXIC EFFECTS OF CADMIUM.

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Lippia alba (Mill.) N.E. Brown is a plant widely used in tropical countries for medicinal purposes. Its essential oil isolated from fresh leaves and stems has antioxidant properties. Because it is well known that Cd induces oxidative damage affecting DNA, the objective of this work was to determine if the essential oil of Lippia alba could prevent the cytotoxic and genotoxic effects of this metal using the Allium cepa assay. Onion bulbs with growing roots were exposed to the essential oil at concentrations ranging from 0.01 to 100 μg/mL in the presence or absence of 200 μM Cd, and incubated for 24, 48 and 72 hours. After treatment, roots were cut and measured for weight and length. Root tips were fixed, hydrolyzed in HCl, stained with acid fuchsin, squashed and examined using light microscopy. The essential oil alone did not produce genotoxic damage when compared to its vehicle control (DMSO), but diminished root weight at the largest tested concentration. In contrast, Cd exposure significantly decreased root growth and mitotic index but increased genotoxic damage such as meiotic cells with chromosome bridges, laggards and fragments. These effects were abrogated by the essential oil in a dose-dependent manner, reaching statistical significance at levels greater than or equal to 10 μg/mL. Taken together, these results suggest that the essential oil of Lippia alba can protect from genotoxic damage induced by Cd (Supported by COLCIENCIAS-CENIVAM, RC 432-2004).

722 TOXICITY OF THE LICHEN XANTHOPARMELIA CHLOROCHROA.

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In the Spring of 2004, approximately 600 elk died in the Red Rim Dailey wildlife area southwest of Rawlins, WY. The etiologic agent was ultimately identified as the vagrant, foliose lichen Xanthoparmelia chlorochroa. This finding stimulated interest in the toxicity of lichen substances as the lichen and one of its major components had been extensively advertised as "herbal Viagra" on the internet. In an effort to identify the active toxin in Xanthoparmelia and elucidate the pathogenetic mechanism of action we exposed sheep and mice to the lichen from the area of the mass mortality and to purified usnic acid. Purified usnic acid produced massive skeletal muscle necrosis between 400 and 600 mg/kg body weight, but was not otherwise similar to the syndrome seen in the elk. Air-dried lichen, collected immediately after the die-off produced a syndrome similar to that seen in the elk. Lichen collected from other areas and seasons produced similar signs, but was markedly less potent. We are currently comparing the chemical profile of the various lichen sources to identify the active toxin(s) responsible for paralysis.

723 SAFETY ASSESSMENT OF MALE ENHANCEMENT DIETARY SUPPLEMENTS.

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Many dietary supplements claim to benefit men seeking to enhance erectile function and performance. These supplements often claim to be safe and effective yet often are adulterated. Dietary supplement manufacturer LifeSpan LLC, Portland, OR, allowed our company to independently assess the safety of their formulation before any human studies were conducted or the product was placed into commerce. The formulation contains 5 vitamins, 4 minerals, 1 amino acid, and 8 botanicals. We characterized each ingredient to create a fingerprint, identified marker compounds for later quality control, inclusive of a panel to determine the presence of erectile dysfunction (ED) API's and 168 other API's that might effect erectile function. One ingredient was an extract that needed characterization and method development and a single-lab method validation. Among assays/tests conducted according to OECD protocols in GLP laboratories, were the following: cytotoxicity; 3 genotoxicity tests – AMES test, mouse peripheral blood micronucleus, chromosome aberrations in Chinese hamster ovary (CHO); acute toxicity (limit test); immortalized human hepatocytes assay of CYP1A2/CYP3A4 enzymes. Since no negative results were reported, the formulation was studied in rats during a 3-month period to assess its aphrodiasic properties. 2 doses (allometrically equivalent & 10 times greater) (N=14/group), given by gavage, evaluated changes in: behavior, BW, water consumption, food intake, cauda epididymal sperm density, male sex hormones, penile size, reproductive organs (testes, epididymis, prostate gland, seminal vesicle), endocrine glands (pituitary gland, adrenal gland), liver, kidney, spleen, and blood/urine chemistries. No significant adverse effects or changes were observed compared to 14 controls. These kinds of toxicology studies should be carried out at a minimum to assess the safety of dietary supplements before beginning human studies, and/or offering them to consumers.

724 THE EFFECT OF HIGH DELTALINE CONCENTRATION ON THE TOXICITY OF METHYLLYCACONITINE IN MICE.

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Larkspurs (Delphinium) are one of the most serious toxic plant problems on foothill and mountain rangelands in the western U.S. Total costs to the livestock industry have been estimated at over $20 million annually. Larkspur plants contain numerous norditerpenoid alkaloids (>18) which occur as one of two types, the MDL-type including deltaline and lycoctonine, and the MSAL-type including methyllycaconitine (MLA) and 14-deacetylnudicauline (14-DAN). The MSAL-type is much more toxic (typically > 20 x). Currently management recommendations for planning grazing schemes are based primarily on MSAL alkaloid concentrations in larkspurs. Delphinium barbeyi is one of the more problematic species of tall larkspur plants due to its high concentration of MLA and 14-DAN. However, the most abundant norditerpenoid alkaloid in most D. barbeyi populations is either deltaline or 14-acetyldictyocarpine (14-ADC), less toxic MDL alkaloids. Although the toxicity of MLA, 14-Dan, 14-ADC, and deltaline has been individually determined, it is not known what synergistic or antagonistic effects the large concentration of deltaline or 14-ADC in the plant has on the toxicity of MLA. Therefore, it is critical to understand what contributions MDL alkaloids have on the overall toxicity of larkspurs. The effect of deltaline on the toxicity of MLA was assessed by comparing the LD50 of these alkaloids administered individually to Swiss-Webster mice versus the co-administration of these alkaloids at three different ratios, 1:1, 1:5, and 1:25 MLA to deltaline. The LD50 for MLA alone was 4.0 ± 0.3 mg / kg B.W. and the LD50 for deltaline alone was 113.3 ± 6.4 mg / kg B.W. The co-administration of MLA and deltaline at 1:1 resulted in an LD50 of 2.7 ± 0.3 mg / kg B.W. The LD50 for the 1:5 group was 2.5 ± 0.2 mg / kg B.W. and the LD50 for the 1:25 group was 1.9 ± 0.1 mg / kg B.W. These results suggest that deltaline has an additive effect on the toxicity of MLA in mice and potentially plays an important role in the overall toxicity of tall larkspur plants in cattle.

725 PUERARIN STIMULATES HEME OXYGENASE-1 EXPRESSION THROUGH PI3K AND NRF-2 PATHWAY.

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Heme oxygenase-1 (HO-1) is a stress response protein and is known to play a protective role against the oxidative injury. These induction of HO-1 gene is primarily regulated at the transcriptional level, and its inducibility by various inducers is

linked to the transcription factor NF-E2-related factor 2 (Nrf2). Puerarin, an isoflavonoid found in Pueraria Radix, has been reported to possess antiatherogenic, antihyperglycemic and antioxidant activities. In the present study, we examined the protective mechanism puerarin on tert-butyl hydroperoxide (t-BHP)-induced oxidative injury in mouse Hepa1c1c7 cells. Puerarin up-regulated HO-1 expression and this expression conferred hepatoprotection against oxidative injury induced by t-BHP. Moreover, puerarin induced Nrf2 nuclear translocation, which was found to be an upstream step of puerarin-induced HO-1 expression, and phosphatidylinositol 3-kinase (PI3K)/AKT activation, of which pathway was involved in induced Nrf2 nuclear translocation, HO-1 expression and hepatoprotection. Taken together, these results suggest that puerarin augments cellular antioxidant defense capacity through induction of HO-1 via the PI3K-Nrf2 signaling pathway, thereby protecting cells from oxidative stress.

726 ATHEROGENIC AND PULMONARY RESPONSE OF APOE- AND LDL RECEPTOR-DEFICIENT MICE TO CIGARETTE SMOKE.

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Previous studies from our laboratory have shown that exposure to sidestream cigarette smoke (SSCS) accelerates atherogenesis in apolipoprotein-E deficient mice. In comparison to apoE-deficient mice, the lipoprotein profile of LDL receptor-deficient mice is closer to that of humans. Furthermore, the lipoproteins in these two strains differ with regard to lipid and apolipoprotein composition and susceptibility to oxidation. To determine if the nature of hypercholesterolemia predisposes to SSCS-mediated promotion of atherosclerosis, we compared the pulmonary and cardiovascular response of apoE- and LDLr-deficient mice to identical SSCS exposure. Female apoE-/- and LDL receptor-/- mice were maintained on a saturated fat diet and exposed to SSCS in whole body exposure chambers for 15 weeks (4 hrs/day, 5 days/week). At sacrifice, the levels of injury markers in bronchoalveolar lavage (BAL) fluids from 6 mice/group and the atherosclerotic lesion formation in 14 mice/group were analyzed. Moderate increases in total BAL cells were noted in the SSCS-exposed groups of both mouse strains but the differences were not statistically significant. Total protein, LDH and cytokine levels in cell-free BAL fluids were also not significantly affected by SSCS exposure in either mouse strain. However, SSCS exposure significantly increased the percent atherosclerotic lesion area covering the aortic intimal surface in apoE-/- (25.3±1.52-Control and 31.9±2.02-SSCS) as well as in LDLr-/- (30.97±1.1-Control and 36.61±1.7-SSCS) mice. In contrast, the serum cholesterol values of SSCS-exposed apoE-/- mice were similar to controls (1255±85 vs 1190±61mg/dl) but increased significantly in SSCS-exposed LDLr-/- mice (998±114 vs 1577±142 mg/dl). It is concluded that SSCS exposure produces similar effects on atherosclerosis despite the significant differences in hypercholesterolemia in apoE-/- versus LDL receptor-/- mice. (Supported in part by Phillip Morris External Research Program).

727 EXACERBATION OF ATHEROSCLEROSIS FOLLOWING EXPOSURE TO VARIOUS COMBUSTION SOURCE PARTICLES.

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Exposure of ApoE knockout ($ApoE^{-/-}$) mice to concentrated ambient particles (CAPs) has been shown to increase arterial plaque area and size. CAPs are a complex aerosol mixture consisting of wind-blown dust, emissions from the combustion of fossil fuels, and secondary transformation products. Studies have yet to determine the contribution of specific sources to CAPs–induced exacerbation of cardiovascular disease (CVD). Studies were initiated to assess the effect of specific combustion source particles on CVD. A 1 month inhalation study was conducted to assess the effects of diesel exhaust (DE) (6h/dy, 5dy/week, 2mg/m3) on atherosclerosis in $ApoE^{-/-}$ mice maintained on either high fat or normal chow diets. Significant increase in plaque area was seen in DE/normal chow group versus the air/normal chow group. No effect on plaque area was observed between the DE/high fat versus air/high fat chow groups. Heart cellularity was also assessed and DE exposure produced a greater decrease in $ApoE^{-/-}$ mice fed normal chow. Subsequent studies examined the ability of equivalent amounts of diesel exhaust particles (DEP), coal fly ash (CFA), residual oil fly ash (ROFA), and Mt. St. Helen's (MSH) volcanic ash to affect plaque area following pulmonary exposure by pharyngeal aspiration (23 ug/mouse/week) of $ApoE^{-/-}$ mice fed high fat or normal chow diets for 3 months. ROFA, DEP, and MSH particles were found to induce acute pulmonary inflammation at 24h post-exposure. Quantitative analysis of plaques demonstrated a significant increase in plaque area within all exposure groups fed normal chow compared to saline exposed controls. No difference versus controls was observed in any of the exposure groups fed the high fat chow. No hierarchy in the ability to increase plaque area was observed among the different combustion particles. These results indicate that exposure to combustion source particles exacerbates atherosclerosis in a manner independent of both inflammation and dietary fat intake. (This abstract does not necessarily reflect EPA policy)

728 INHALED NICKEL NANOPARTICLES INDUCE SYSTEMIC INFLAMMATION AND ACCELERATE ATHEROSCLEROTIC PLAQUE FORMATION IN APOPROTEIN E (APOE) KNOCKOUT MICE.

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It has been suggested that inhaled nanoparticles (NPs) may induce toxic effects in the cardiovascular system as well as in the pulmonary system. One of the proposed mechanisms of this systemic toxicity is that inhaled NPs can induce systemic inflammation, which might ultimately contribute to the development of various adverse cardiovascular heath effects including atherosclerosis. The objective of this study was to determine the long-term cardiovascular effects of inhaled nickel (Ni) NPs by inducing systemic inflammation and ultimately accelerating atherosclerosis in a hyperlipidemic mouse model. Male $ApoE^{-/-}$ mice, 5-month old, were exposed to either filtered air or 100 $\mu g/m^3$ of Ni NPs (n=8/group) in whole-body exposure chambers for 5h/d, 5d/w, for 5 months. To track systemic inflammation, blood samples were taken every 3 weeks and analyzed for selected inflammatory markers including C-reactive protein (CRP), ilterleukin-6 (IL-6), vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and E-selectin. Atherosclerotic plaques in the aortic arch were characterized by a non-invasive ultrasound scanning technique, every 6 weeks. Among the inflammatory markers measured, CRP had shown the most significant difference between the control and exposed group at the 8th week of exposure ($P<0.05$). It was also shown that plaque formation in the aortic arch was accelerated in the Ni-exposed mice, but the significant difference did not appear until the 11th week of exposure. These results clearly showed that inhaled NPs could induce systemic inflammation and accelerated atherosclerosis in a sensitive animal model. Since systemic inflammation preceded the accelerated plaque formation in this study, it may also indicate a causal association between systemic inflammation and atherosclerosis. Further studies will be needed to confirm the mechanisms in which systemic inflammation could contribute to atherosclerosis.

729 HEALTH EFFECTS OF TRAFFIC RELATED PARTICULATE MATTER.

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Traffic generated ultrafine particulates (UF) may play a major role in the development of adverse health effects. However, little is known about harmful effects caused by recurring exposure. We hypothesized that repeated exposure to PM results in adverse pulmonary and systemic toxic effects. Fischer F344 rats, with a mild pulmonary inflammation at the onset of exposure, were exposed for 4 weeks, 5 days/week for 6 hours a day to a) ambient particles at an urban background, b) diesel engine exhaust or c) PM near a motorway (<2.5 μm). UF which are largely present in diesel soot may enter the systemic circulation and could trigger systemic effects. Hence, we assessed vascular function in arteries as well as inflammatory response in different brain regions. We focused on diminished endothelial function, reduced fibrinolysis, and increased blood coagulation. All effects were investigated 24 hours after termination of the exposure. There were indications of mild oxidative stress and inflammation as indicated by respectively reduced levels of anti-oxidants and increased neutrophils in the bronchioalveolar lavage fluid of rats exposed to PM at the background location. Furthermore, we detected a decrease in pro-inflammatory cytokine levels in the brain of these PM exposed animals. Exposure to PM from a motorway impaired fibrinolysis and there were no signs of inflammation in the lung or systemic system. Exposure to diesel engine exhaust resulted in an oxidative stress response and impaired fibrinolysis and coagulation. Pro-inflammatory cytokine levels were decreased in some regions of the brain but increased in the striatum implying that exposure to diesel engine exhaust may aggravate neurological impairment. The studies presented in this paper suggest that exposure to traffic related PM can mediate changes in the vasculature and brain of healthy rats. To what extent these changes may contribute to chronic neurodegenerative or vascular diseases is at present unknown.

730 WILL THE GASEOUS DIESEL EXHAUST SYNERGISTICALLY EXACERBATE CONCENTRATED AMBIENT PARTICLES INDUCED PROGRESSION OF ATHEROSCLEROSIS PLAQUE IN APOE-/- MICE?

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Atherosclerosis is one of the most important contributors to cardiovascular disease, a leading cause of mortality and morbidity in developed countries. Epidemiology studies have shown that polluted ambient air, composed of both particulate and gaseous phases, is an important risk factor for atherosclerosis. Some human and animal studies have shown that the ambient particulate matter (PM) exacerbates the atherosclerosis progression. However, since the gas phases of the air pollution have been denuded from exposure, these studies may have underestimated the adverse health effects of realistic ambient air exposure. In this study, we seek to understand the effects of gaseous phases air pollution, either alone or interacted with particulate matter, on enhancing atherosclerosis chronically. Gaseous components of diesel exhaust were used in this study. Atherosclerosis-susceptible mice (ApoE-/-) were exposed sub-chronically to either 1) filtered ambient air, 2) concentrated ambient particles (CAPs), 3) gaseous diesel exhaust (GDE), 4) CAPs + GDE, or 5) whole diesel exhaust (DE) for 6hr/d, 5d/w, for 6 months. Every four weeks, bloods were withdrawn from facial vein, and serums were analyzed for cytokine activation, such as vCAM1, CRP etc. At 3 and 6months of exposure, the atherosclerotic lesion size (n=10 per group per timepoint) in braciocephalic artery, left common carotid artery, aortic root and abdominal aorta was assessed via both histology stains and a serial non-invasive ultrasound biomicroscopy. The effects on lungs, heart and brains were also investigated. Preliminary analysis showed that CAPs (210 μg/m3)+GDE (equivalent to 509 μg/m3 DE) produced greater atherosclerotic lesions than CAPs alone. The results of this study could answer the question of whether gas phase pollutants in the ambient air, by itself or in an additive/synergic effect with particulate matter, may exacerbate the progression of atherosclerosis in susceptible populations.

731 ALTERATION OF CARDIAC FUNCTION BY URBAN AND RURAL CONCENTRATED AMBIENT PM.

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Numerous epidemiological studies have shown that airborne fine particulate matter (PM2.5) is a causative factor in increased cardiac mortality via impairment to the autonomic nervous system (ANS) and cardiac function. However, the reported effects of PM exposures on cardiac function vary from many studies. This may result from different sources of PM components. As part of the HEI's NPACT initiatives (National Particle Composition Toxicological initiatives), we are conducting subchronic CAPs inhalation studies in mice in diverse U.S. locations in order to identify the PM components that are most responsible for acute and cumulative cardiovascular system health effects. During the first study, we have simultaneously compared the effects of CAPs in Sterling Forest to those encountered in Manhattan, NYC, where there are substantial increments of Ni and elemental and organic carbon in the ambient PM in addition to the long-range transport PM that is present in both locations. Sixteen ApoE-/- mice (n=8/group) were exposed to comparable concentrations of CAPs (avg. 170±130 g/m3) or filtered air at each location, 6 h/d, 5 d/w, for 6 months. Electrocardiogram (ECG), Heart Rate (HR), deep body temperature, and activity counts data were constantly sampled for 20 seconds/5 minutes throughout the experiment using implanted DSI telemetry transmitters. Heart Rate Variability (HRV) analysis was conducted using conventional methods (SDNN; RMSSD; PSD, power spectral analysis). The results show that HR decreased significantly in mice after two months CAPs exposure at the Sterling Forest site between 22:00 and 6:00, which is consistent with the results of our previous studies; while no decreasing trend of HR was found in CAPs exposure mice at Manhattan site. Body temperatures were stable for mice at both sites. No significant changes were found in HRV analysis at in the initial 2-months of the study at both sites. Our results demonstrate that subchronic CAPs exposures at locations with different particle compositions produce different effects on cardiac function in ApoE-/- mice.

732 FINE PARTICULATE MATTER EXPOSURE EXACERBATES ATHEROSCLEROSIS IN GENETICALLY SUSCEPTIBLE (APOE KNOCKOUT) MICE.

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Particulate matter (PM) exposure disrupts cardiac function and worsens cardiovascular disease (CVD) but the mechanisms are not well understood. PM exposure induces inflammation in blood vessels and exacerbates atherogenesis. In this study ApoE-/- mice were exposed to concentrated ambient fine PM (CAPs) with an MMAd ≤ 2.5 μm. Control mice were exposed to purified air. Factors relevant to mechanisms of atherogenesis and the development of cardiovascular heart disease were measured. The thickness of artery walls was measured in anesthetized mice after 0, 2 and 4 months of exposure to CAPs using an ultrasound microprobe with a resolution of 30 μm. C-reactive protein (CRP), matrix metalloprotease (MMP-9), platelet activation inhibitor (PAI-1), adhesion molecules (sICAM-1 and sVCAM-1) and e-selectin were measured in plasma. Compared to controls, CAPs exposure significantly reduced MMP-9 and increased PAI-1 which could modify normal vascular injury repair and platelet function, respectively. There were no significant differences in e-selectin, ICAM-1 or VCAM-1 between the groups. Arterial wall thickness (AWT) increased in both control and CAPs-exposed mice over the 4-month exposure however arterial walls in CAPs-exposed mice were significantly thicker than those of controls. Although mean concentrations of plasma CRP were not different in air-exposed and CAPs-exposed mice, CRP concentration was significantly and linearly related to AWT. There was a significant interaction between CRP and CAPs exposure such that the slope of the CRP/AWT relationship for CAPs-exposed mice was significantly greater than that for air-exposed mice. Additional samples are now being analyzed to test the hypothesis that oxidative stress in vascular tissue induced by CAPs exposure initiates and exacerbates atherosclerosis. This study demonstrates that chronic PM exposure can accelerate atherosclerosis in genetically susceptible populations.

733 ULTRAFINE PM AFFECTS CNS INFLAMMATORY PROCESSES AND MAY ACT *VIA* MAP KINASE SIGNALING PATHWAYS.

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Exposure to ambient PM increases the risk of cardiopulmonary disease. Adverse effects have also been seen in the brains of mice after PM exposure. Inhaled, nanosize particles can penetrate the lungs and be deposited in extra-pulmonary tissues and may reach the brain either by transport along the olfactory nerve or by penetration of a blood brain barrier. In this study the activities of some signaling pathways that mediate brain inflammatory responses were up-regulated in mice exposed to concentrated PM. We also looked at biomarkers of oxidative stress and tissue injury. Apolipoprotein E knockout (ApoE-/-) mice were exposed to concentrated "ultrafine" PM (UF) with an aerodynamic diameter of less than 0.18 μm in central Los Angeles. Mice were exposed to two different concentrations UF CAPs for 5 hr per day, 3 days per week for about 8 weeks; ~25 μg/m3 and ~200 μg/m3. Control mice were exposed to purified air. Mice were euthanized 24 hr after the last exposure and tissue was stored at -80C until analyzed. There was clear evidence of aberrant immune activation in the brains of exposed animals as judged by a dose-related increases of two key nuclear transcription factors, NF-κB and AP-1 which are involved in inflammatory responses. We also saw increased levels of glial fibrillary acidic protein (GFAP), suggesting that astroglia were participating in this response. Levels of basal and phosphorylated (activated) MAP kinases that activate NfκB and AP-1 were assayed by Western blotting. There were small increases in phosphoylated ERK-1 and p38 and a small decrease IκB. However these changes were not statistically significant. This study confirms that PM exposure affects CNS inflammatory processes and examines some possible links to MAP kinase signaling pathways.

734 CHRONIC EXPOSURE OF APOLIPROTEIN E KNOCKOUT MICE TO FINE PARTICLES INDUCED HEART RATE CHANGES BUT NOT CHANGES IN TIME DOMAIN MEASUREMENTS OF HEART RATE VARIABILITY.

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Epidemiological studies have shown a link between ambient air pollution exposure and cardiovascular disease risk. The hypothesis of this study is that chronic exposure of atherosclerotic-prone mice to concentrated ambient particulates results in accelerated atherogenesis. Male Apoliprotein E knockout (ApoE-/-) mice were exposed to fine particles, diameter <2.5μm, near the interchange of three major freeways in Riverside, California. The mice received an average particle concentration of approximately 200μg/m3 for five hours per day, four days per week for six months. Animals were maintained on purified air in the vivarium between exposures. Surgical implantation of telemetry devices prior to exposures allowed monitoring of electrocardiogram and body temperature in 10 of the animals; five mice

from the purified air group and five mice from the fine group. Heart rate and heart rate variability were obtained through analysis of the electrocardiogram data. Electrocardiograms were monitored during the five hour exposure period and the overnight purified air periods. Data collected during the exposure period shows a decrease in heart rate of animals exposed to concentrated particulate matter compared to animals exposed to purified air. During non-exposure periods, no difference in heart rate between the two groups was observed. Preliminary analysis suggests that heart rate variability, measured as the standard deviation of normal beat to beat intervals, was not different between the two groups. Further study is needed to determine if adverse physiological effects result from the decreased heart rate.

735 EXPOSURE TO PARTICULATE MATTER POTENTIATES INSULIN RESISTANCE ASSOCIATED WITH MACROPHAGE ACCUMULATION IN ADIPOSE TISSUE.

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Background: Recent studies have implicated air-borne particulate matter <2.5µm ($PM_{2.5}$) in diabetes, insulin resistance, and metabolic syndrome in humans. We investigated the effects of $PM_{2.5}$ exposure in diet-induced diabetic mice.
Methods: Male C57BL/6 mice, fed with high fat chow for 3 months, were exposed to concentrated ambient $PM_{2.5}$ or filtered air (FA) in whole-body exposure chambers for 5 months (Oct. 2006 to Mar. 2007) in Tuxedo, NY (n=8, 6 h/d, 5 d/wk).
Results: The mean (SD) daily $PM_{2.5}$ concentration was 7.9 (5.0) µg/m^3 at the study site in Tuxedo, 72.7 µg/m^3 in the exposure chamber (approximately 9-fold concentration from ambient Tuxedo levels), and 13.0 µg/m^3 the mice were actually exposed when normalized over the 5-month period. Insulin/glucose ratios, glucose tolerance test, and weights were identical prior to exposure; $PM_{2.5}$ exposure worsened fasting glucose levels (161±4 vs 139±6 mg/dl, p<0.05 vs FA), insulin concentrations (0.6±0.1 vs 0.2±0.1 ng/ml, p<0.01 vs FA), hyperglycemia after glucose loading (432±47 vs. 250±15 mg/dL at 120 minutes, p<0.001 vs FA) and insulin levels (0.6±0.1 vs 0.6±0.1 ng/ml) while vasodilator response to insulin was attenuated in preconstricted aortic segments. $PM_{2.5}$ exposure significantly increased adipose tissue macrophage infiltration by F4/80 staining, caveolin and TOPRO3 colocalization (21±3% vs 9±4%, p<0.01 vs FA), and adipocyte size (mean area 1000 µm^2 vs 800 µm^2, p<0.01 vs FA) by H & E staining in the epididymal fat pads. Phox (p22phox, p47phox, and p91phox) family gene expression was also increased in stromal vascular cells in the $PM_{2.5}$ group compared with FA group.
Conclusion: Exposure to $PM_{2.5}$ exaggerates diabetes, potentiates insulin resistance, and induces macrophage infiltration in adipose tissue that is associated with augmented gene expression in NAD(P)H oxidase subunits, suggesting a novel link between air pollution and metabolic syndrome.

736 ADVERSE CARDIOVASCULAR EFFECTS WITH ACUTE PARTICULATE MATTER AND OZONE EXPOSURES: THE ROLE OF TOLL-LIKE RECEPTOR 4.

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OBJECTIVES: Elevations in ambient particulate matter (PM) levels are associated with cardiovascular morbidity and mortality in numerous epidemiology studies. Fewer studies have examined the role of copollutants, such as ozone (O3), in this association. Furthermore, the mechanisms by which PM affects cardiac function remain unclear. We hypothesize that PM and ozone as copollutants induce adverse cardiovascular effects in mice, and that the Toll-Like Receptor 4 (TLR4), significantly involved in innate immunity, is a candidate susceptibility gene.
STUDY DESIGN: Radiotelemeters were implanted to measure heart rate (HR) and HR variability (HRV) parameters, then C3H/HeJ (C3), C57Bl/6J (B6) and C3H/HeOuJ (OUJ) inbred mouse strains were exposed to 3 different daily exposures of filtered air (FA), carbon black particles (CB), or a combination of O3 and CB (O3CB).
RESULTS: HR and HRV were significantly changed in all strains due to CB alone or following O3 relative to FA. The TLR4-mutant C3 mice uniquely exhibited persistent elevations in HRV measured on the morning after exposure to O3CB. Strain-specific effects of CB with O3 pre-exposure included markedly attenuated HRV responses in B6 mice relative to the other strains. Oxygen pulse in B6 mice was also significantly elevated following CB exposure alone or in combination with O3, which was not evident in the other strains.
CONCLUSIONS: These results suggest that the TLR4 pathway, among other factors, modulates cardiac recuperation following acute air pollution exposures.

737 DIESEL EXHAUST EXPOSURE AUGMENTS ENDOTHELIN CONSTRICTOR SENSITIVITY AND DIMINISHES ACETYLCHOLINE MEDIATED DILATION IN RAT CORONARY ARTERIES.

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Epidemiological evidence clearly shows an association between air pollution and increased incidence of both acute and chronic cardiovascular disease. The pollutant diesel exhaust (DE) has been shown to increase endothelin (ET) synthesis and sympathetic nervous system activity; therefore, these events may be key to DE-induced cardiovascular sequelae. We hypothesized that acute exposure to moderate levels of DE would augment ET-1 constrictor sensitivity and diminish agonist mediated dilation. Rats were exposed to 5 hrs of DE (300 µg/m^3) or Air. Coronary arteries were obtained after DE exposure to measure ET-1 constrictor responses and agonist-induced dilator responses. In isolated septal coronary arteries (inner diameter = 180 ± 11 µm) from Air and DE rats, DE augmented ET-1 constrictor responses and nitric oxide (NO) synthase (NOS) inhibition (L-NNA, 100µM) augmented ET-1 responses only in the Air to normalize response between groups. Acetylcholine (ACh)-mediated dilation was diminished in arteries from DE exposed rats but was augmented by L-NNA treatment such that dilation was not different between groups in the presence of L-NNA. In summary, increased constrictor sensitivity to ET-1 and decreased endothelium-dependent dilation could impair oxygen delivery to the heart in certain pathological states. DE inhalation increases systemic generation of reactive oxygen species (ROS). Elevated ROS may uncouple NOS thereby diminishing basal NO release to enhance ET-1 constriction. Uncoupled NOS may also produce endothelium-derived vasoconstrictors that antagonize ACh-mediated dilation. Inhibition of NOS therefore restores agonist-dependent dilation in coronary arteries following DE exposure. These changes in coronary vasoreactivity may contribute to the detrimental cardiac effects of DE and the increased incidence of cardiac events observed following this exposure.

738 INHALED COMPLEX COMBUSTION EMISSIONS UPREGULATE TRANSCRIPTION AND ACTIVITY OF SYSTEMIC MATRIX METALLOPROTEINASE-9 (MMP9): EVIDENCE IN MURINE AND HUMAN MODELS.

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Air pollution is associated with acute and chronic adverse human health effects related to atherosclerotic pathologies. MMP9 has a crucial role in the progression and ultimate degradation of vascular lesions and polymorphisms of MMP9 are highly associated with increased incidence of myocardial infarction. Following evidence that gasoline exhaust emissions induce transcription of MMP9 in the aortas of apolipoprotein E knockout mice, we sought to test the integrity and translational validity of this marker across varying pollutant atmospheres and also in human samples. ApoE mice were exposed by inhalation to primary gaseous components of gasoline engine emissions, namely carbon monoxide (CO; 8 and 80 ppm), nitrogen dioxide (NO_2; 0.2 and 2.0 ppm) and nitric oxide (NO; 17 ppm). Other groups of mice were also exposed to biogenic secondary organic aerosols, coal emissions, and diesel exhaust. Results of MMP9 mRNA changes, as assessed by real time PCR, indicate a strong role for freely-diffusible components such as CO and NO, but also some degree of induction by pollutant components that do not penetrate the lung in meaningful levels (PM, NO_2). However, no single component produced effects as great as the whole exhausts, suggesting that systemic vascular effects are driven by the sum of components. We then assayed plasma MMP9 protein in humans and found a significant upregulation of MMP9 (p=0.0003) following diesel exhaust exposure compared to air control exposures. These results strongly suggest that systemic effects are not initiated by any one pollutant component and imply that accurate assessment of air pollution health effects must consider the cumulative impact of whole exhaust constituents. This abstract was funded by NIH 5F32ES015404-02, DOE DE-FC04-96AL76406, EPA CF 826442-01-1 and does not necessarily reflect EPA policy.

739 GASOLINE ENGINE EMISSIONS EXPOSURE RESULTS IN UPREGULATION OF ENDOTHELIN-1 – MEDIATED VASCULAR MATRIX METALLOPROTEINASE ACTIVITY.

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Cardiovascular disease is currently the leading cause of death in the US, with atherosclerosis being responsible for approximately half of these deaths. Epidemiological studies report a positive correlation between environmental air pollution and in-

creased rates of cardiovascular morbidity and mortality. Remodeling of arterial extracellular matrix is a crucial step in the progression of atherosclerosis. We have previously reported that expression of vascular remodeling proteins, matrix metalloproteinases (MMPs), and vasoactive peptide endothelin-1 (ET-1), as well as reactive oxygen species (ROS) are found upregulated in ApoE-/- mice exposed to gasoline engine emissions (GEE). Thus, we tested the hypothesis that GEE exposure-induced ET-1 mediates ROS, and expression of MMPs in the vasculature. To determine the role of ET-1 in vascular ROS and MMP expression, ApoE-/- mice, on a high fat diet, were treated with either ETA receptor antagonist, BQ-123, (100 ng/kg/day via osmotic minipump) or vehicle, and concurrently exposed via inhalation for 6 h/d, for a period of 7 d to either GEE (60 μg PM/m3) or filtered air. Exposure to GEE resulted in a significant increase in aorta ET-1, MMP-2, and MMP-9 mRNA, as determined by real time PCR. Western blots and in situ zymography confirmed an increase in MMP-2 and -9 proteins and activity, respectively, in GEE-exposed aortas. Dihydroethidum staining and TBARs analysis of GEE-exposed aortas were indicative of elevated ROS. Effects of GEE on mRNA, protein, and TBARS levels were all significantly attenuated by BQ-123 treatment. Such findings suggest that exposure to gasoline emissions results in elevated vascular ET-1, which mediates, at least in part, upregulation of molecular pathways involved in vascular remodeling, inflammation, and the progression of atherosclerosis. Funded by NIH 5F32ES015404-02 (AKL) and EPA CF 826442-01-1(MJC).

740 CARBON NANOTUBE ACUTE LUNG EXPOSURE INDUCES PLASMINOGEN ACTIVATOR INHIBITOR 1.

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Plasminogen activator inhibitor (PAI)-1 is an inhibitor of fibrinolysis, the physiological process involved in blood clot degradation. Systemic release of PAI-1 has been associated with ischemic cardiovascular disease and poor prognosis of acute lung injury. We evaluated the induction of PAI-1 in the lung and systemic circulation of C57BL/6J mice exposed to high dose (40 μg) purified single walled carbon nanotubes (SWCNT, CNI, Inc.) or multi walled CNT (MWCNT, provided by Prof. Endo, Shinshu University, Japan) by pharyngeal aspiration. CNTs were dispersed by sonication in a dispersion solution (DS) containing mouse serum albumin and DSPC. Mice were sacrificed 4hr post-exposure and lung tissue, bronchoalveolar lavage (BAL), and blood were collected for biochemical and gene expression assessment. CNT exposure caused increased PAI-1 (~5-fold) gene expression in the lung while no increase was found in the whole blood. PAI-1 protein levels were significantly increased in the lungs of both CNT-treated groups (3.57±0.42 ng/ml DS; 7.45±0.68* SWCNT; 8.16±0.89* MWCNT, *$p<0.05$). A similar trend was demonstrated for the plasma PAI-1 protein levels (1.95±0.19 ng/ml DS; 2.36±0.57 SWCNT; 2.84±0.38 MWCNT) as well as plasma PAI-1 activity (0.33±0.10 DS; 0.64±0.16 SWCNT; 0.80±0.16* MWCNT, *$p<0.05$). The PAI-1 activation was accompanied by induction of lung inflammation including increased influx of neutrophils in the BAL, increased BAL LDH and albumin and elevated lung tissue gene expression of numerous inflammatory genes such as (IL-1β, MIP-2, MCP-1, and IL-6). Furthermore, acute CNT exposure induced MIP-2 and IL-1β gene expression in the blood cells from the systemic circulation. All these effects were more prominent and consistent in mice exposed to MWCNT compared to the mice exposed to SWCNT. In conclusion, CNT-induced acute lung inflammation was associated with a systemic response which may lead to disturbances in the coagulation cascade and vascular endothelial function.

741 THE AORTA, BUT NOT LUNG OR HEART, IS THE TARGET OF SUBCHRONIC DIESEL-INDUCED INJURY AND INFLAMMATION.

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Cardiovascular abnormalities have been noted in individuals exposed to diesel exhaust (DE) or traffic air pollution; however, the mechanisms are unknown. We postulated that long-term episodic exposure of rats to DE would differentially affect the three target organs: lung, heart, and aorta. We exposed healthy male Wistar Kyoto rats (12-15 wks old), nose-only, to either fresh air or bulk DE from the tail pipe of 30 kW Deutz engine (2.0 mg/m3), 5 h/d, 1 d/wk for 16 wks. We analyzed gene expression by real-time PCR which reveal contributions of inflammation, oxidative stress, microvascular thrombosis and vasoconstriction. Minimal pulmonary inflammation was noted following DE exposure. Gene expression in the lung and the heart did not change except for a ~2-fold induction in atrial and brain natriuretic peptide mRNA in the lung. However, remarkable changes were noted in the aorta. While the inflammation and oxidative stress genes (MIP-2, HO-1) were induced ~3-fold over control, the most remarkable induction was noted for markers of thrombosis (tissue plasminogen activator inhibitor (~22-fold), tissue plasminogen activator (~9-fold), thrombomodulin (~4-fold), and van Willebrand Factor (~3-fold). The expressions of endothelin (ET) and its receptors were also induced (ET, ~9-fold; ET receptor A, ~3-fold and receptor B, ~2-fold) but not angiotensin-2. Surprisingly, the expression of natriuretic peptides was inhibited in the aorta following DE exposure. These data demonstrate that aorta is the target of DE-induced injury. Episodic DE exposure produces remarkable vascular abnormalities while sparing the heart and the lung. The aortic injuries and thrombosis produced by ambient levels of DE in healthy individuals may secondarily lead to cardiac physiological alterations. (Does not reflect US EPA policy). Supported in part by EPA NCBA/SEE Program and EPA/UNC Cooperative Agreement #CT829471.

742 CHANGES IN ENDOTHELIAL TUBE CELL-CELL BORDERS IN RESPONSE TO DIESEL EXHAUST PARTICLES.

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Several epidemiological studies link exposure to diesel exhaust particles (DEP) with adverse cardiovascular events. Other studies show that inhaled DEP gain access to the bloodstream. The mechanisms for how DEP gain access to the capillary lumen and how they cause cardiovascular problems are unclear. To elucidate possible mechanisms, we have used human umbilical vein endothelial cells (HUVEC) plated on plastic and Matrigel as in vitro and organ culture model systems. DEP collected from a Japanese diesel engine (gift of Dr. Sagai), were diluted and sonicated (to about the size of PM2.5) in 0.05% TWEEN/PBS for 5 min. DEP at concentrations of 1, 10, and 100 in 1 ml medium were added to 150,000 cells in monolayer culture or as capillary endothelial tubes. After 24 or 48 hr exposures, proliferation and cytotoxicity assays were used to examine cell viability of monolayer cultures, TUNEL assays were used to determine the extent of apoptosis of treated capillary tubes, and confocal microscopy was used to evaluate the redistribution of VE-cadherin in the cell-cell borders of tubes. At 100 ug/ml DEP, over 80% of the cells die within 48 hr. Cells treated with 1 ug/ml DEP doubled every 24 hr, as do untreated cells. However, 10 ug/ml DEP delayed doubling. TUNEL assays indicated that some cell death was occurring at this concentration. In addition, Z-stacks of confocal images revealed that DEP not only accumulated on the surface of capillary tubes, but also penetrated into the lumen. VE-cadherin was observed to redistribute in response to DEP. Endothelial cell borders were slightly affected at 1 ug/ml, more affected at 10 ug/ml, and were almost totally disrupted at 100 ug/ml. Our results suggest that DEP gain access to the bloodstream by altering the endothelial cell junctions.

743 EXACERBATION OF ATHEROSCLEROSIS BY URBAN AND RURAL CONCENTRATED AMBIENT PM.

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A number of epidemiologic studies have demonstrated a link between particulate matter (PM) and cardiovascular morbidity and mortality. We have previously shown acute and cumulative effects of daily inhalation exposures to concentrated ambient particles (CAPs) in Sterling Forest, NY in a mouse model of atherosclerosis. Although PM from sources rich in nickel and vanadium were found to be responsible for changes in heart rate (HR) and heart rate variability (HRV), the sources responsible for atherosclerosis changes were not clearly demonstrated. In the HEI's NPACT initiatives (National Particle Composition Toxicological initiatives), we are conducting subchronic CAPs inhalation studies in mice in diverse U.S. locations in order to identify the PM components that are most responsible for acute and cumulative cardiovascular system effects. In this study, we simultaneously compared the effects of CAPs in Sterling Forest to those encountered in Manhattan, where there are substantial increments of Ni and elemental and organic carbon in the ambient PM in addition to the long-range transport PM that is present in both locations. Male mice lacking apolipoprotein, 3-month old, n=12/group, on normal rodent chow, were exposed to CAPs, simultaneously, in our Sterling Forest laboratory in Tuxedo and in Mt. Sinai School of Medicine in Manhattan for 6 hr/d, 5d/w, for 6 months (avg. conc. 170±130 μg/m3). Atherosclerotic lesions in the brachiocephalic and left common carotid arteries were measured by serial non-invasive ultrasound biomicroscopy at 3 and 6 months of exposure. The plaque size in both the brachiocephalic and left common carotid arteries was significantly increased at 3 months of CAPs exposure. At this time point,

comparable effects were seen in both locations. Our results indicated that CAPs induced early progression of atherosclerosis appears to be less affected by PM compositions than other cardiac endpoints (e.g. HR and HRV).

744 LIPOIC ACID PREVENTS VASCULAR DYSFUNCTION AND INFLAMMATION IN PARTICULATE AIR POLLUTION EXPOSURE.

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Background: Recent studies have suggested a link between inhaled fine particulate matter (diameter <2.5μm, $PM_{2.5}$) exposure in urban areas in the exacerbation of vascular diseases. We hypothesized that antioxidant lipoic acid (LA) prevent vascular dysfunction in air pollution.
Methods: Male C57BL/6 mice, fed with high fat chow for 3 months, were exposed to concentrated ambient $PM_{2.5}$ or filtered air (FA) in whole-body exposure chambers for 5 months (Oct. to Mar. 2007) in Tuxedo, NY (n=16, 6 h/d, 5 d/wk). During the last 2 months, LA (50 mg/kg, or saline) was also administered intraperitoneally daily.
Results: The mean (SD) daily ambient $PM_{2.5}$ concentration was 7.9 (5.0) μg/m3 at the study site in Tuxedo, 72.7 $\mu g/m^3$ in the exposure chamber (approximately 9-fold concentration from ambient Tuxedo levels), and 13.0 $\mu g/m^3$ that the mice were actually exposed when normalized over the 5-month period (within EPA national standard 15.0 $\mu g/m^3$). LA treatment corrected hyperglycemia and hyperinsulinemia (258 ± 30 vs. 432 ± 47 mg/dl and 0.3 ± 0.1 vs. 0.5 ± 0.1 ng/ml, respectively, $p<0.05$) in response to intraperitoneal glucose loading and improved endothelial relaxation response to acetylcholine in aortic segments when compared with $PM_{2.5}$ group. LA prevented increases in circulating TNF-α, IL-6, ICAM-1, E-selectin, and PAI-1, decreased adipocyte size (H&E staining) and prevented macrophage infiltration (F4/80 staining and caveolin and TOPRO3 co-localization) in epididymal fat associated with $PM_{2.5}$ exposure.
Conclusion: Exposure to $PM_{2.5}$ exaggerates diabetes, potentiates insulin resistance, vascular dysfunction, and induces macrophage infiltration in adipose tissue. LA can prevent vascular dysfunction and inflammation induced by $PM_{2.5}$ exposure.

745 EFFECTS OF FRESHLY-GENERATED LOW- AND ULTRALOW-SULFUR FUEL EMISSION AEROSOLS IN INSULIN-RESISTANT, OBESE RATS.

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We hypothesize that inhaled ambient ultrafine (UF) particles can induce oxidative stress-related injury of target cells in the lungs and cardiovascular system, thus providing a plausible mechanism for adverse effects observed in humans with type II diabetes-related cardiovascular disease. We measured pulmonary and cardiovascular endpoints related to inflammation and oxidative stress in obese diabetic rats with atherosclerotic lesions after exposure to freshly-generated UF/fine particle-containing aerosols generated from low- and ultralow-sulfur Diesel fuel in a mobile laboratory. Lean (6-8 months) and obese (9-10 months) JCR rats were exposed (6 hrs/day; 1 or 4 days) in compartmentalized whole-body chambers to aerosols that were sampled from the engine exhaust plume (0.4-1.8 x $10^6/cm^3$; 10-15 nm) while driving on a freeway. Blood, bronchoalveolar lavage (BAL) fluid, and several tissues were collected after exposure. BAL levels of plasminogen activator inhibitor (PAI)-1 and interleukin-1β were slightly but significantly increased by exhaust exposure. For PAI-1, these increases were larger after 4 days of exposure. We did not find consistent exposure-related changes in plasma inflammatory mediators. Obese JCR rats had higher leptin levels in BAL and blood and were hyperlipidemic, which raises the possibility that oxidative defense mechanisms are different in lean and obese rats. These results show that small oxidant stress-related changes occur after acute exposure to real-world particles in an animal model relevant to the study of particle effects in susceptible humans.
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746 REPEATED EXPOSURE EFFECT OF TRAFFIC RELATED AIR POLLUTANTS ON THROMBOGENICITY.

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Exposure to traffic related air pollution (particulate matter (PM)) has been associated with exacerbation of respiratory and cardiovascular diseases. Recently, we established that the procoagulant state in lungs after acute exposure to PM is related to inflammation and could be explained by the ratio of tissue factor (TF) and thrombomodulin activities. The aim of the present study was to evaluate the adverse effects of repeated PM-exposure on the inflammation induced prothrombotic state. Fischer F344 rats, with a mild pulmonary inflammation at the onset of exposure, were exposed for 4 weeks, 6 hours/day for 5 days, followed by 2 days of rest to either diesel engine exhaust, PM near a motorway, or particle-free atmosphere (control) in two different experimental settings. Tissue factor (TF) activity and thrombin generation were assesed in lungs 24 hours after the last exposure. TF activity was not altered upon recurring exposure to diesel engine exhaust or PM. Exposure to diesel exhaust decreased the endogenous thrombin potential (ETP: 46±12%, $p<0.05$) and peak height (65±18, $p=0.05$) for lung homogenates compared to control animals (ETP: 55±9, peak height: 78±14) for analysis in the presence or absence (data not shown)of additional TF. Exposure to PM near a motorway had no influence on the ETP and peak height for lung homogenates, whereas the lag time was prolonged (105±7% vs. 99±7%, $p<0.05$). The combination of these results suggest that repeated exposure to PM has no influence on the prothrombotic state within lung tissue. In contrast, repeated exposure to diesel engine exhaust even decreased the overall thrombogenic potential, suggesting either desensitization to recurring exposure, or preconditioning of lung cells to prolonged toxic stimulation. These findings stress the importance of repetative exposure studies, in addition to acute exposures, for delineating the exact consequences of particulate matter in vivo.

747 PARTICLE-INDUCED RELEASE OF CYTOKINES IN MONO- AND CO-CULTURES OF PRIMARY LUNG AND HEART CELLS.

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Epidemiological studies have linked exposure to particulate air pollution (PM) with both pulmonary and cardiovascular morbidity and mortality. Inflammation is considered as a key event in the development of these effects. The ultrafine size fraction of PM (< 100 nm) has gained special interest as the small particle size may favour translocation to extra-pulmonary organs. PM-associated cardiac effects may be caused by translocated particles, particle components and/or mediators related to inflammation from the lung. Primary epithelial lung cells (type 2) and mono– and co-cultures of cardiac cells (cardiomyocytes and cardiofibroblasts) isolated from rats, were exposed (0-400 μg/ml) to ultrafine carbon black particles (Printex 90, Degussa, Germany) for 20 h. Release of interleukin (IL)-6, tumor necrosis factor-alpha (TNF-α) and IL-1β was measured by ELISA. Cardiac cells were also exposed to medium from particle-exposed lung cells. A particle-induced increase in IL-6 release was observed in all cell cultures except the cardiofibroblasts. Independently of exposure, a synergistic release of IL-6 was observed in co- versus mono-cultures of cardiac cells. A particle-induced increase of IL-1β was observed in lung cell cultures and sporadically in co-cultures of cardiac cells, whereas TNF-α was not detected in any of the studied cell cultures. Compared to direct particle exposure the release of pro-inflammatory cytokines by cardiac cells was strongly enhanced when exposed to lung cell-conditioned medium. The experiments demonstrate a potential for ultrafine carbon black particles to induce a pro-inflammatory response in epithelial lung cells, in cardiomyocytes and in co-cultures of cardiomyocytes and cardiofibroblasts. A strong enhancement of these responses, observed in cardiac cell cultures exposed to medium from particle-exposed lung cells, indicates an importance of pulmonary inflammation in particle-induced cardiac cell responses.

748 ACUTE EXPOSURE TO PARTICULATE MATTER (PM) IN A RAT MODEL OF HEART FAILURE.

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Human exposure to ambient PM is linked to cardiovascular morbidity and mortality. This association strengthens in people with preexisting cardiopulmonary diseases—especially heart failure (HF). To better characterize the cardiovascular effects of PM, we developed a model of accelerated HF in Spontaneously Hypertensive Heart Failure (SHHF) rats. Rats (male, 110d) were implanted with radiotelemeters to monitor electrocardiogram (ECG), heart rate (HR), blood pressure (BP), and core temperature (T_{co}), and infused with the beta-adrenergic agonist isoproterenol (ISO, n=10; 2.5mg/kg/d s.c.) or saline (n=8) via osmotic pump for 28 days. ISO induced immediate hypotension and hypothermia, and a delayed tachycardia relative to control. BP returned to baseline after 12d, but HR stayed elevated during infusion. After removal of ISO pumps, rats experienced a second 12d hypotensive

episode as well as persistent depressions in HR and pulse pressure. Enhanced respiratory pause remained elevated 1 and 2 weeks after ISO. 18d post ISO, rats were exposed on two successive days via nose-only inhalation to filtered air or HP12 (5h/d; 12 mg/m^3)—a metal rich oil combustion-derived PM. PM exposure significantly increased pulmonary lactate dehydrogenase and serum HDL cholesterol only in ISO-treated rats. ISO treatment alone elicited ischemic myocardial lesions and elevations in cardiac mass. Only ISO-treated rats exposed to PM had significantly increased cardiac mass relative to saline-treated rats exposed to either air or PM. There was a trend towards increased incidence and frequency of arrhythmias among rats exposed to PM and ISO combined. Based upon our preliminary findings, PM may exacerbate ISO-induced cardiac toxicity. *(Abstract does not reflect EPA policy; this research was supported by EPA CT82947101.)*

749 DIFFERENTIAL CARDIAC ARRHYTHMIA PROFILES IN HYPERTENSIVE AND NORMAL RATS AFTER EMISSION SOURCE PARTICULATE EXPOSURE.

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Exposure to combustion-derived fine particulate air pollution is associated with increased cardiovascular morbidity and mortality. These effects are especially conspicuous in individuals with pre-existing cardiovascular disease including hypertension. PM exposure causes altered cardiac rhythm often manifested as ST depression, altered ventricular repolarization, and reduced heart rate variability. However, PM-induced dysrhythmias observed in humans have not been readily reproduced in rodent models. We tested the hypothesis that the Spontaneously Hypertensive (SH) rat, a hypertension-prone strain, will be more susceptible to the development of PM-induced arrhythmia than its corresponding normotensive background control, the Wistar Kyoto (WKY) rat. Rats were exposed once by inhalation (nose-only) for 4 hours to residual oil fly ash (ROFA), an emission source particle rich in transition metals, or air, and then sacrificed 1 or 48 h post exposure. SH rats exposed to ROFA had greater pulmonary injury and neutrophil infiltration, and higher levels of the serum acute-phase marker C-reactive protein than similarly exposed WKY rats 48 h post exposure. In addition, only the ROFA-exposed SH rats had shorter ST, QT, and RR interval length relative to air exposed animals during exposure, suggesting that the hypertensive state may result in proarrhythmogenic changes in the myocardium of the SH rats. Interestingly, ROFA exposure in the WKY rats, but not SH rats, caused a reduction in the low frequency (LF) power spectra, an indicator of sympathetic tone. These results suggest that ROFA exposure exacerbates myocardial vulnerability in SH rats while causing some level of autonomic dysregulation in WKY rats (This abstract does not reflect EPA policy; funded in part by EPA CT82947101 & UNC T32-ES07126).

750 DOSE INDEPENDENT CHANGES IN HEART RATE AND HEART RATE VARIABILITY FOLLOWING INHALATIONAL EXPOSURE TO DIESEL EXHAUST.

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Measures of heart rate (HR) and heart rate variability (HRV) have been frequently altered in association with exposures to ambient particulate matter air pollution and are often considered markers of autonomic tone. As diesel exhaust (DE) is a large proportion of urban ultrafine particulate, we measured HR and HRV in a mouse model of cardiovascular disease, the apolipoprotein E deficient (apoE-/-) mouse. Male apoE-/- mice aged 13 months were implanted with electrocardiogram (ECG) radiotelemetry devices and exposed to 1 week of filtered air (FA) followed by 8 weeks inhalation of freshly generated, diluted and aged DE at one of three concentrations determined by $PM_{2.5}$ mass: 100 (n = 9), 200 (n = 4) or 400 μg/m^3 (n = 8), allowing each animal to act as its own control. ECG data were analyzed for HR and the HRV time domain measures of standard deviation between normal to normal beats (SDNN), the root mean square difference between normal to normal beats (rMSSD) and frequency domain parameters. The frequency domain was further divided into the very low frequency, low frequency and high frequency. There was no dose response for any of the measurements evaluated in this study. For all three doses, there was a similar decrease in HR of approximately 100 beats per minute compared to FA. There was also a consistent increase in the SDNN and rMSSD in all three doses. There was no consistent change in the frequency domain parameters with the exception of the low and total frequencies which both exhibited a delayed increase in the two higher doses only. These results suggest that there is no dose response relationship between DE inhalation and HR and HRV parameters. Despite the lack of dose response, a replicable relationship is found in response to DE which can support the use of ECG findings as a biomarker of exposure and may suggest a mechanism that is not dependent on the dose of DE. Funded by NIEHS R01 ES013434.

751 ALTERATIONS OF HEART RATE VARIABILITY AND CARDIOVASCULAR RISK FACTORS IN RATS EXPOSED TO COARSE AND FINE PARTICULATE MATTER.

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Previous epidemiological studies have suggested that coarse particulates (PM2.5-10) may have comparable effects with fine particulates (PM2.5). In this study, we compared the cardiovascular toxicity between fine and coarse particles in spontaneously hypertensive rats (SHR). Male SHRs were implanted with radiotelemeters at the age of 11 weeks. After recovery, each animal was first intratracheally instilled with phosphate buffered saline (PBS) as control, then with PM2.5-10 (200μg, n=7) or PM2.5 (200μg, n=7) at the same weekday and the same hour of the day in the following week. These particles were collected from a traffic busy area. After exposure, animals were kept in a controlled environment, and ECG signals were collected continuously for 72 hours. We used GEE model to investigate the effects of particle-induced alterations of heart rate variability (HRV) including ANN, SDNN and RMSSD. Three other groups of non-implanted SHRs were instilled with PBS (n=8), PM2.5-10 (200μg, n=8) or PM2.5 (200μg, n=8) at the same age. Animals were sacrificed 24 hours after instillations, and blood samples were analyzed for fibrinogen, nitric oxide (NO) and C-reactive protein (CRP). The above parameters were compared between fine and coarse particle groups. Significant increases of fibrinogen and CRP, and decrease in NO were observed 24h after instillation of both PM2.5-10 and PM2.5. Borderline increases of ANN were also observed up to 72h after instillation of both PM2.5-10 and PM2.5. Interestingly, SDNN and RMSSD demonstrated a borderline increase in PM2.5 instillation, but they were both decreased in PM2.5-10 exposure. Our study found that the cardiovascular toxicity of coarse particles was comparable to that of fine particles. The different manifestations of HRV between coarse and fine particles suggest the components of coarse particles responsible for the toxicity need further study.

752 ACUTE EFFECTS OF TOBACCO, INCENSE, AND CANDLE SMOKE ON PORCINE BLOOD GASES AND CARDIOVASCULAR FUNCTION.

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Cigarette, incense, and candle smoke each contain many different chemicals, but all of these smoke sources contain carbon monoxide. We hypothesized that if acute exposure to all sources of smoke caused similar changes in carboxyhemoglobin and cardiovascular function, then carbon monoxide may be an important determinant of smoke-induced effects. Male pigs (20-40 kg) were exposed for 30 minutes sequentially to sham, candle, incense and tobacco smoke with at least 3 days washout between exposures. Blood gases, blood pressure (oscillometry), cardiac function (echocardiography) and endothelial function (flow-mediated dilation or FMD) were then examined 30 minutes post-exposure. Consistent with known effects of nicotine, tobacco smoke significantly increased systolic and diastolic blood pressure (n=3 pigs; 25% and 40% increase; p=0.0001 and 0.017 in Bonferroni posteriori test after repeated measures ANOVA, respectively) compared to sham-exposure. In contrast, candle and incense smoke exposure had no effect on blood pressure (n=8/group). Carboxyhemoglobin increased 52% and methemoglobin decreased 20% in incense- compared to sham-exposed pigs (p=0.0001 and 0.017, respectively). Similar trends in blood gases were observed after tobacco and candle smoke exposure, but were not significant. Incense smoke significantly decreased FMD (75% decrease; p=0.004), while the decrease in FMD after tobacco and candle smoke exposures (both approximately 45%) were not significantly different from sham exposure. Therefore, the results of this study are consistent with the hypothesis that carbon monoxide from smoke plays a role in impairing endothelial function, but not blood pressure or cardiac function. However, other chemicals common to tobacco, incense and candle smoke may also play a role in this impairment. Since endothelial dysfunction is a strong predictor of morbid cardiovascular events in humans, further studies are needed to clarify the risk posed by acute exposure to smoke from a variety of sources.

753 CHANGES IN CARDIAC PHYSIOLOGY AFTER CHRONIC EXPOSURE TO CONCENTRATED AMBIENT PARTICLES IN AGED RATS.

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Previous research has shown that exposure to ambient particulate matter can lead to or worsen cardiac morbidity and mortality in the elderly. To investigate the relationship between chronic exposure to concentrated ambient particles (CAPs) in

rats, 13-month-old male Sprague Dawley rats were exposed to ultrafine particles (UFP) with a mass median diameter <0.18μm, or purified air for nine months in Riverside, California. Average concentration of the UFP was ~100μg/m3. Telemetry devices that were surgically implanted prior to exposures allowed monitoring of electrocardiograms, blood pressure (BP) and body temperature in the ultrafine and purified air groups. Heart rate (HR), heart rate variability (HRV), respiratory rate, interbeat interval, and Q-A intervals were obtained through analysis of the telemetry data. Preliminary analysis of our data shows an acute depression of BP during and immediately after exposure to UFP compared to the purified air group. Small changes in HR, HRV, respiratory rate, interbeat interval, and Q-A intervals were observed, but were not statistically significant. Rats have a significant diurnal pattern with depressed HR and BP during their period of inactivity, particularly the evening hours. Previous studies in our laboratory suggested that the diurnal pattern was blunted in rats exposed to CAPs sub-chronically. It will be necessary to perform a time-series analysis of the data set before drawing additional conclusions; however the observed changes in BP do not appear to be progressive.

754 PATHWAY AND SPECIES DIFFERENCE IN THE BIOACTIVATION OF TRICYCLIC ANILINES CATALYZED BY P450 ENZYMES.

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Covalent binding of reactive metabolite to biological macromolecules, such as proteins and DNA, is a known mechanism of toxicity. Anilines are known structures which could potentially cause toxicity via metabolic activation. In this study, a tricyclic aniline-containing compound, 8-amino-1-(4-fluorophenyl)-4,5-dihydro-1H-benzo[g]indazole-3-carboxamide, was found to form protein-like conjugates in the presence of a series of cysteine-containing trapping agents (from cysteine to GSH, to an 11-amino acid peptide) with liver microsomes or S9. The bioactivation pathway has been elucidated via isolation and identification of glutathione-conjugates and other key metabolites by NMR and LC/MS. P450 enzymes involved in bioactivation have been identified using recombinant CYPs. It has been found that this tricyclic aniline structure undergoes aromatic oxidation to form otho-hydroxy aniline followed by further oxidation to 1,2-iminoquinone reactive metabolites. Species differences and regioselective metabolism via individual P450 enzymes have been observed between rat and human. Human P450 enzymes (CYP3A4, 1A2 and 2D6) exclusively oxidize at C-7 followed by addition of GSH at C-6. Whereas rat P450 enzymes oxidize at either the C-7 or C-9 positions, furthermore to form GSH conjugates at C-6 or C-7. Rat CYP2C (2C6, 2C11 and 2C12) appears to favor the C-7 oxidation, while rat CYP3A (3A1 and 3A2) appears to prefer the C-9 oxidation. These data may provide some guidance on the appropriate risk assessment and interpretation of metabolism and toxicity translated from experimental animals to human in drug development.

755 CYTOCHROME P450 2E1 PHENOTYPING IN HUMANS: DOSE-DEPENDENT METABOLISM OF CHLORZOXAZONE MAY CAUSE UNRELIABLE RESULTS.

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Cytochrome P450 2E1 (CYP2E1) is an important enzyme in the biotransformation of many organic solvents, industrial chemicals, therapeutic drugs and endogenous substances such as ketones and aldehydes. Chlorzoxazone (CZX) is commonly used for in vivo phenotyping of CYP2E1. Conventionally, a fixed dose of 500 mg CZX is given orally and the parent substance and its major metabolite 6 hydroxychlorzoxazone (HCZX) are measured in plasma after 2 h. The phenotype is expressed as the metabolic ratio (HCZX/CZX). Many factors, such as fasting, obesity, liver dysfunction, ethanol consumption and drug intake, have been shown to influence CYP2E1 activity and may thus contribute to intra and inter individual variability. However, the appropriateness of the dose has rarely been discussed. We tested different fixed doses of CZX (250, 500 and 750 mg) in the same volunteers, and also a weight adjusted dose (10 mg/kg body weight). Further, the possibility to use saliva samples instead of plasma was investigated.

The in vivo experiments, in total 356 experiments in 50 subjects, show that the metabolic ratio decreases with increasing administered as well as absorbed dose, the latter expressed as the sum of CZX and HCZX in plasma at 2 h. The increase in metabolic ratio becomes pronounced at a total concentration below 100 μM. We could neither detect CZX nor HCZX in the saliva samples.

In conclusion, the metabolic ratio is dose-dependent. We therefore suggest that a weight-adjusted dose of 10 mg CZX/kg bw is used to assess the CYP2E1 phenotype. Further, we suggest that the metabolic ratios be disregarded when the sum of parent and metabolite (CZX+HCZX) is below 100 μM in plasma sampled at 2 h after intake.

The study was supported by the Swedish Council for Working Life and Social Research (Grant 2001-2242).

756 EFFECT OF TROGLITAZONE ON INDUCTION OF CYTOCHROME P450 1A1 AND BENZO[A]PYRENE-DNA ADDUCT FORMATION IN HEPA-1C1C7 CELLS.

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Troglitazone, an antidiabetic drug is selectively activates peroxisome proliferator-activated receptor (PPAR), affected the expression of several of these genes. Several peroxisome proliferators enhance cytochrome P450 1A1 (CYP1A1) activity but the mechanisms involved remain unknown. In this study, we examined the effect of the PPAR-g agonist, troglitazone, on CYP1A1 gene expression and activity via the arly hydrocarbon receptor (AhR)-independent pathway. Troglitazone increased gene expression of CYP1A1 mRNA and also increased CYP1A1-specific 7-ethoxyresorufin O-deethylase activity. Moreover, concomitant treatment with troglitazone and GW9662, a PPAR-g antagonist, markedly reduced the troglitazone-inducible EROD activity. We found that a putative peroxisome proliferator response element (PPRE) between -521 and -500 is located in the CYP1A1 gene promoter. Their inactivation by deletion mutagenesis suppressed the inductive effect of troglitazone on CYP1A1 promoter activation. Electrophoretic mobility shift assay revealed that troglitazone induced the activation of the PPAR-g to a form capable of binding specifically to the PPRE sequence of the CYP1A1 gene promoter. Furthermore, troglitazone increased the formation of the Benzo[a]pyrene-DNA adduct. Overall, our results suggest that troglitazone induced CYP1A1 enzyme activity and gene expression through PPAR-g activation, and may be involved in carcinogen biotransformation.

757 A COMPARATIVE STUDY FOR LIVER-TOXICITY INDUCED WITH ACETAMINOPHEN AND ITS REGIOISOMER, N-ACETYL-M-AMINOPHENOL IN RATS.

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It is well known that over dose of acetaminophen (4'-hydoxyacetanilide, APAP) causes liver toxicity in human and animals by reactive metabolite mediated by cytochrome P-450, but not N-acetyl-m-aminophenol (3'-hydroxyacetanilide, AMAP), which is a regioisomer of APAP, at least in mice. However, the formation of reactive metabolites in mice has been reported in both compounds. The reason why AMAP can't cause hepatotoxicity is not well known. Thus, in the present study, mechanism for hepatotoxicity induced with APAP but not AMAP were analyzed using rats and rat hepatocytes treated with both the compounds on the basis of metabolic activation via P-450 and elimination via conjugates formations.

Serum alanin aminotransferase (ALT) was significantly elevated in rats orally treated with 1000 mg/kg of APAP but not AMAP. The decrement of rat hepatocytes-ATP activity, which is an indicator for cytotoxicity, was obviously observed at 30 mM in APAP using rat primary culture hepatocytes. After pre- and co-treatment with borneol, which inhibits glucuronidation activity, APAP decreased ATP activity in rat hepatocytes at more than 3 mM but AMAP decreased ATP activity in rat hepatocytes at only 30 mM. Further, by pre- and co-treatment of buthionine-S,R-sulfoximine (BSO), which inhibits glutathione (GSH) biosynthesis, ATP activity largely decreased by exposed to APAP but not AMAP in rat hepatocytes. These in vitro results reflected the results of rat in vivo study. Thus, the reason why no hepatotoxicity was observed in rats treated with AMAP might be related with the balance between activation by P-450 and inactivation by conjugations. Furthermore, this approach by comparing inspections in vitro and in vivo studies is thought to be very useful to evaluate the mechanism of hepatotoxicity induced by reactive metabolites.

758 PARTICIPATION OF MICRORNA IN THE REGULATION OF HUMAN CYP3A4 EXPRESSION.

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MicroRNA (miRNA) is a group of small non-coding RNAs that regulates gene expressions through translational repression or mRNA cleavage. More than 500 miRNAs have been identified in human and some miRNAs are characterized in the function of various biological processes such as cell proliferation, differentiation and apoptosis. The human cytochrome P450 (CYP) 3A4 is a member of CYPs

and plays a key role in metabolizing various endogeneous compounds and xenobiotics including therapeutic drugs. CYP3A4 enzyme exhibits inter-individual variation at the activity level. Single nucleotide polymorphisms (SNPs) of CYP genes have been analyzed but only few SNPs have been associated with observed inter-individual differences. An extremely long 3'-UTR is peculiar to CYP3A4. In this study, we investigated whether human CYP3A4 expression was correlated with miRNA expression.
The RNA fractions (large and small molecules) were prepared from human liver tissues of 30 individuals. The potential miRNAs interacting with CYP3A4 and related nuclear receptor genes were listed using miRBase database. The expression levels of CYP3A4, PXR, CAR and miRNAs were analyzed by the real-time reverse-transcription polymerase chain reaction method (RT-PCR) using TaqMan Gene Expression and MicroRNA Assays. The expression level of CYP3A4 correlated with those of both PXR and CAR ($p<0.01$). Among 14 miRNAs investigated in this study, miR-148a level was correlated with CYP3A4 ($p<0.05$) and miR-224 level was inversely correlated with both CYP3A4 ($p<0.05$) and PXR ($p<0.01$). These results suggest that miR-148a and miR-224 participate differently in the regulation of CYP3A4 expression. miR-148a might affect on CYP3A4 expression and miR-224 might affect on PXR or both PXR and CYP3A4 expressions.

759 INDUCTION OF P4504A1 ACTIVITY AND PEROXISOMAL PROLIFERATION BY LAMBDA-CYHALOTHRIN.

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For risk assessment, it is of prime interest to obtain relevant information on pharmacokinetics including metabolism of pyrethroids in animals and humans. Relatively little is known of the effect of pyretroids on hepatic microsomal drug-metabolizing function. The objective of the present study is twofold: (a) to establish if lambda-cyhalothrin, Type II pyretroid insecticide, interacts with hepatic microsomal lauric acid hydroxylase activity, which is closely associated with the P4504A subfamily of hemoproteins, and (b) to determine whether P4504A and peroxisomal proliferation are coaffected by lambda-cyhalothrin. Animals were treated with lambda-cyhalothrin (4 and 8 mg/kg/day orally for 6 days); control animals received orally corn oil. Pyrethroid-treated and control animals were sacrificed 24 hours after the last administration and livers were removed. The livers were individually homogenized. Aliquots of whole homogenate were reserved for peroxisomal and mitochondrial enzyme determinations and microsomal pellets were prepared and stored at –80C for determinations of 11- and 12-hydroxilations of lauric acid. Following treatment with lambda-cyhalothrin (4 and 8 mg/kg) there were highly significant increases in both hepatic peroxisomal cyanide-insensitive palmitoyl-CoA oxidation (36 and 60%, repectively) and total (peroxisomal plus mitochondrial) carnitine acetyltransferase activity (28 and 57%, respectively). Treatments of rats with lambda-cyhalothrin gave rise also to a concentration-dependent increase in hepatic microsomal lauric acid hydroxylase activity (68 and 107%, respectively, for lauric acid 11-hydroxylase activity; 26 and 86%, respectively, for lauric acid 12-hydroxylase activity). These results provided evidence that lambda-cyhalothrin caused induction of P4504A subfamily and also produced peroxisome proliferation. This work has been supported by projects
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760 ACTIVATION OF ARISTOLOCHIC ACID TO MUTAGENIC METABOLITES BY HUMAN CYP1A1, 1A2 AND 3A4.

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Aristolochic acid (AA) has been implicated as the cause of Chinese herb nephropathy and endemic (Balkan) nephropathy (EN) and associated urothelial cancers. Numerous biotransformation enzymes may play a role in AA activation and/or detoxification, including cytochrome P450s 1A1 and 1A2 (CYP1A1/1A2), NAD(P)H:quinone reductase (NQO1), xanthine oxidase (XO), and prostaglandin H synthase. To elucidate the roles of CYP enzymes in the activation of AA, we conducted an Ames mutagenicity assay with various cDNA expressed human CYPs. In this study, we utilized the TA 98 *Salmonella typhimurium* strain to evaluate mutagenicity of AA (156, 520, and 666 ug/plate) in the presence or absence of three CYP activating systems (purified human enzymes CYP1A1, CYP1A2, and CYP3A4 plus NADPH and an NADPH regenerating system). Aflatoxin B1 (AFB), which is activated by both CYP1A2 and 3A4, served as a positive control. After a 48-hr incubation at 37C, revertant colonies were counted. There was a dose-dependent increase in number of revertant colonies for each of the three activating systems. As shown previously, AA appeared to be weakly mutagenic in the absence of an activating system. Mutagenicity, signified by at least a doubling of revertants over control, was not observed at the lowest AA dose in the CYP1A2 plates but was noted at the two higher doses. Mutagenicity occurred at all AA doses in the CYP1A1 and 3A4 plates. The slope of the dose response curve was noticeably steeper for CYP1A1 and 3A4 when compared to CYP1A2. These data demonstrate that human CYP1A1, 1A2 and 3A4 are capable of activating AA to mutagenic metabolites and suggest that, of these enzymes, CYP3A4 may be the most effective P450 for AA activation. However, this does not rule out the potential dual role of and or all of these P450s in also forming 'detoxification' products of AA, as demonstrated previously for both CYP1A2 and 3A4 toward AFB.

761 AROMATASE EXPRESSION AND TRANSCRIPTIONAL REGULATION BY BENZO(A)PYRENE AND INHIBITOR FADROZOLE.

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We hypothesized that a target for reproductive or developmental dysfunction caused by benzo(a)pyrene (BaP) exposure could be the P450 enzyme aromatase (CYP19). In fish, there are two isoforms of aromatase, a predominantly ovarian form, CYP19A1, and a brain form, CYP19A2. CYP19 expression was measured by *in situ* hybridization following BaP exposure (0, 10, 100 μg/L waterborne for 10 or 15 days) in *Fundulus* adults, juveniles and embryos. The CYP19A1 expression was significantly decreased in the BaP treated group in the ooplasm of stage I and II oocytes in the 3 month old, but not adult, *Fundulus*. Expression of CYP19A2 was decreased compared to controls in brains of both embryos and adults. In order to compare the BaP-mediated inhibition with that of a known aromatase inhibitor, fish were also exposed to fadrozole (20 μg/L and 100 μg/L). Fadrozole did not decrease the mRNA expression in the brain and ovary of embryos and/or adult *Fundulus*. However, aromatase enzyme activity was significantly decreased in fadrozole-treated adult ovary and brain tissues. In addition, to further understand the mechanism underlying the BaP-mediated decrease in CYP19 expression, we cloned the promoter regions of the *Fundulus* aromatase genes and found putative response elements including CRE, AhR and ERE. Ongoing work is studying the transcriptional regulation of these CYP19 promoters in CHO-K1 cells. These studies will provide a greater molecular understanding of the mechanisms of action of BaP and its potential to impact reproduction or development. Supported by NIEHS R01ES012710.

762 CLONING, EXPRESSION AND PARTIAL CHARACTERIZATION OF CYTOCHROME P450 3A37 FROM TURKEY LIVER THAT OXIDIZES AFLATOXIN B1.

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Turkeys are one of the most sensitive animals to the mycotoxin aflatoxin B1 (AFB1). We have previously shown that this sensitivity is due, at least in part, to efficient hepatic cytochrome P450 (CYP)-mediated bioactivation of AFB1 coupled with deficient glutathione S-transferases that can detoxify the exo-AFB1-8,9-epoxide (AFBO) so produced. In turkey liver, AFB1 bioactivation is mediated largely by human CYP1A2 homologue(s) and to a lesser extent to CYP3A4 homologues. We recently cloned CYP1A5 from turkey liver, and the heterologously expressed protein had high AFB1 epoxidation activity, and also produced the detoxified, soluble metabolite AFM1. To identify other possible AFB1 bioactivating CYPs, we used 3'- and 5'- rapid amplification of cDNA ends (RACE), to amplify a CYP3A4 homologue, CYP3A37 (Genbank DQ450083) from turkey liver. This gene has an ORF of 1512 bp, and the protein is predicted to be 504 amino acids with 97% identity to chicken CYP3A37. A truncated construct of the turkey CYP3A37 gene with 11 amino acids deleted from the hydrophobic N-terminal region was heterologously expressed in Escherichia coli. The expressed protein oxidized AFB1 to form two metabolites: exo-AFBO (Km = 16.79 ± 5.1μM; Vmax = 1.17 ± 0.082nmol/min/nmol P450) and the detoxified metabolite aflatoxin Q1 (AFQ1) (Km = 23.15 ± 10.66; Vmax = 2222.01 ± 1377.09nmol/min/nmol P450). Comparing these kinetic paramters with those previously reported for CYP1A5, it appears that CYP3A37 has greater activity toward AFB1 epoxidation. However, a firm determination of their relative importance in turkey liver awaits immunoinhibition studies. Supported in part by National Research Initiative grant 2006-04819 from the USDA Cooperative State Research, Education, and Extension Service Animal Genome program.

763 TEMPORAL CHANGES IN MOUSE CYP mRNA WITH CLASSIC INDUCERS.

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Assessment of profile changes of CYP induction in acute rodent preclinical studies through genomic approach has been emphasized in recent years due to understanding mechanisms of gene transcriptional activation and advanced molecular technologies. There are still some puzzles around transcriptional alterations down to the final enzyme activity changes. The purposes of the present study are to provide patterns of temporal changes up to 8 days of Cyp 1a1, 1a2 and 2b10, 3a11 and 4a10 at mRNA, protein and activity levels in a mouse study, and discuss advantages and shortcomings of genomic approach when used in preclinical settings. Male CD-1 mice were treated daily for 8 days with phenobarbital, dexamethasome, clofibrate, beta-nephthoflavone and control vehicles. Liver samples were obtained after 0.5, 1, 2, 4, and 8 days of dosing for RNA and microsomal preparation. RNA were extracted with Qiagen kits and converted to cDNA with TaqMan® Reverse Transcription Reagents Kits. PCR was conducted using TaqMan Universal PCR Master Mix Kits. Conventional enzyme activity and Western blot was performed for the same genes. It was found that excellent correlation present between genomic and protein/activity approaches. Transcriptional changes often occurred earlier and folds changes in earlier time points (up to 1 day) were relatively higher than activity level in cases of 2b10 and 1a1. Fold changes between 2 and 8 days for genomic and conventional assays are much more paralleled. It was concluded that genomic approach is a useful tool to study Cyp induction in mouse models, and is more appropriate and relevant to activity level when treatment with duration of two or more days was applied.

764 TESTICULAR CYP1B1 REGULATION IN MOUSE MA-10 AND RAT R2C LEYDIG CELLS.

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CYP1B1 is expressed at a relatively high level in extrahepatic tissues such as adrenal and testis. Previously, we reported that rat testicular CYP1B1 protein expression is developmentally regulated and is suppressed after hypophysectomy. Pituitary hormones such as luteinizing hormone (LH), follicle stimulating hormone or prolactin positively regulate constitutive CYP1B1 protein expression in rats. In the present study, we investigated the regulation of testicular CYP1B1 expression by steroidogenic hormones and modulators of protein kinase and estrogen receptor in vitro. Two Leydig cell lines, mouse Leydig tumor cells (MA-10) and rat Leydig tumor cells (R2C) were used for CYP1B1 studies in vitro. Immunoblots were carried out to detect CYP1B1 protein in Leydig cell microsomes. RT-PCR assay was performed for CYP1B1 and LH receptor mRNA analysis. Protein kinase A (PKA) activity in the cell lysates was determined using an enzyme-linked immunosorbent assay. Leydig cells were treated with LH, 8-Br-cAMP (PKA activator), H89 and Rp-CPT-cAMP (PKA inhibitors), Go 6983 (PKC inhibitor), estradiol benzoate, ICI 182,780 (estrogen receptor antagonist), flutamide (anti-androgen) or anastrozole (aromatase inhibitor). A high constitutive expression of CYP1B1 protein was detected in rat Leydig cells R2C. In MA-10 cells, CYP1B1 mRNA expression was induced by LH and 8-Br-cAMP in a dose- and time-dependent manner with concomitant stimulation of PKA activity. PKA inhibitors (H89 and Rp-CPT-cAMP) suppressed basal CYP1B1 mRNA expression in a dose- and time-dependent manner. H89 or Rp-CPT-cAMP was able to inhibit the 8-Br-cAMP- and LH-mediated increase of CYP1B1 mRNA expression and PKA activity when MA-10 cells were co-treated with LH or 8-Br-cAMP, and PKA inhibitors. The results suggest that LH regulates CYP1B1 through the cAMP-PKA pathway in MA-10 cells. CYP1B1 mRNA expression was suppressed after estradiol benzoate treatment in MA-10 cells. ICI 182,780 treatment failed to alter estradiol-mediated CYP1B1 mRNA expression. Studies are ongoing to explore the CYP1B1 regulation in rat R2C cells.

765 HUMAN METABOLISM *IN VITRO* OF ATRAZINE AND CHLOROTHALONIL.

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Atrazine is an herbicide widely used for control of broadleaf and grassy weeds in corn, sugar cane, turf grass, etc. Although it has been studied for a considerable time, little information is available on its metabolism in humans. The current study was carried out to investigate in vitro human metabolism of atrazine using pooled human liver microsomes (pHLM) and also a number of cytochrome P450 (CYP) isoforms. Four metabolites of atrazine were identified as follows: desethylatrazine (DEA); desisopropylatrazine (DIA); hydroxyatrazine (HA); methoxyatrazine (MA). HA has not previously been shown to be a human metabolite of atrazine. The apparent Km and Vmax values for DEA and DIA were 79.71 ± 9.5 μM and 684.17 ± 20.5 nmol/min/mg protein and 440.6 ± 40.2 μM and 662.46 nmol/min/mg protein, respectively. With pHLM, DEA and DIA were the major metabolites with concentration of atrazine of 2.5 - 50 μM. However, the production of HA and MA was increased at higher concentrations of atrazine (50 - 500 μM). The results from testing 13 human CYP isoforms for atrazine metabolic activity showed that CYP1A2 and CYP2C19 were the major isoforms for DEA and DIA production, respectively. CYP2C18 and CYP4A11 were the major isoforms responsible for HA production, while no specific isoform(s) was found for MA production. In preliminary studies, the metabolism of the fungicide, chlorothalonil, by pHLM was also studied. These studies showed that chlorothalonil is a substrate for pHLM, producing at least one metabolite.

766 ADRENOCORTICAL TOXICITY FOLLOWING CYP11A1 METABOLISM OF A KINASE INHIBITOR.

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Background: A kinase inhibitor intended for use in the treatment of cancer induced atrophy of the adrenal cortex (z. fasciculata and z. reticularis) in mouse, rat, dog and monkey. Radio-labeled drug accumulated in the rat adrenal gland to concentrations 30-fold greater than found in the liver. Binding to adrenal proteins and toxicity were abrogated by aminobenzotriazole (ABT), a suicide inhibitor of cytochrome P450 (CYP) mediated drug metabolism. The objective of this study was to determine whether toxicity was mediated by CYP metabolism in mouse or human adrenal tissue.
Methods: Cultures of mouse Y-1 and human H295R adrenocortical cell lines were exposed to increasing concentrations of the kinase inhibitor in the presence or absence of general CYP inhibitors (ABT, ketoconazole: KTC), a specific inhibitor of CYP11A1 (aminoglutethimide: AGT) or CYP11B1/2 (metyrapone: MET), or CYP inducers (ACTH, forskolin: FSK). Cells were also treated following > 90% inhibition of each step in steroidogenesis by CYP-selective siRNAs. Endpoints included cell protein, ATP, caspase 3/7 activity, and HPLC assay of steroid metabolism.
Results: The most sensitive measure of toxicity in vitro was caspase activation, indicating induction of apoptosis. Toxicity was observed in Y-1 cells, after only two hours exposure to concentrations equivalent to those measured in vivo in the adrenal gland. Toxicity and metabolism were induced significantly (> 100%) by ACTH and abrogated dose-dependently by ABT, KTC and AGT, but not by MET. In contrast, H295R cells were unaffected by the drug, and caspase activity was induced modestly by forskolin, but not by ACTH. Steroid synthetic activity was slightly greater in H295R than in Y-1 cells. siRNAs against CYP11A1 alone significantly ameliorated caspase activation.
Conclusions: Metabolism of a kinase inhibitor by CYP11A1 induced apoptosis in mouse, but not in human adrenal cortex-derived cells, indicating a species difference in drug metabolism by this enzyme.

767 METABOLISM OF DI- AND TRI-HALOACETIC ACIDS IN RODENTS AND HUMANS: ISOFORM SPECIFICITY AND IMPACT OF GENETIC POLYMORPHISMS.

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Halogenated acetic acids (HAAs) are formed during the disinfection of drinking water. Several tri- and di- HAAs are rodent carcinogens and may be associated with adverse pregnancy outcomes in humans. For most HAAs, the systemic bioavailability and extent of internal exposure is regulated by liver metabolism. Di-HAAs such as dichloroacetic acid (DCAA) are metabolized by the cytosolic enzyme GST-zeta. Tri-HAAs can be metabolized by both cytosolic and microsomal enzymes with the latter pathway increasing with bromine substitution. Microsomal metabolism of tri-HAAs proceeds by reductive dehalogenation, which is stimulated under nitrogen headspace and 2% oxygen atmospheres. Eadie-Hofstee plots for the consumption of the parent tri-HAA suggests multiple P450 isoenzymes are responsible, particularly under N2 and 2% oxygen atmospheres. Incubation of rodent microsomes with carbon monoxide or diphenyleneiodonium greatly reduces the metabolism of tri-HAAs. Inhibitors of CYP 2E1 and 3A4 appear to be effective at reducing microsomal metabolism of tri-HAAs. However, tri-HAA microsomal metabolism was similar between wild-type and 2E1 KO mice. In contrast, immuno-inhibition studies using anti-CYP 2E1 and human microsomes significantly reduced tri-HAA metabolism, suggesting interspecies differences in the isoform specificity. With regard to di-HAAs, GST-zeta is known to be polymorphic in humans with four alleles characterized. Single nucleotide polymorphisms (SNPs) in the GST-Z regulatory region have also been identified. Using archived blood samples from volunteers who participated in a previous DCAA pharmacokinetic study, we compared the

GST-Z genotype with selected DCAA pharmacokinetic parameters such as total body clearance (Clb). No correlation was observed between variation in GST-Z genotype and Clb. However, a small but significant difference in Clb was observed in volunteers with a specific SNP in the GST-Z regulatory region. Supported by EPA grants R825954 and R828044.

768 MUTATIONS IN THE PXR GENE AFFECT BASAL EXPRESSION AND INDUCIBILITY, BUT DO NOT PREVENT THE ABILITY OF SULFORAPHANE TO INHIBIT LIGAND BINDING TO PXR.

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Sulforaphane (SFN) is a naturally occurring isothiocyanate that strongly up-regulates NRF2/Keap1 responsive genes. We have previously shown that SFN also acts as an antagonist to the Pregnane Xenobiotic Receptor, PXR, a promiscuous receptor important in the expression of many genes involved in xenobiotic metabolism and transport, especially CYP3A4, the most abundant CYP in human liver and one that contributes to the metabolism of >50% of pharmaceuticals. Induction of CYP3A4 is a major cause of drug-drug interactions. SFN inhibited PXR-mediated induction of CYP3A4 mRNA for up to 24 hours after removal of SFN from the medium in LS180 cells. Thus, we examined residues in PXR that may covalently react with SFN. Mutation of each of the cysteines in the binding pocket did not affect ability of SFN to inhibit ligand activation of PXR. Two polar residues identified as potentially important to PXR ligand binding are H407 and Q285; mutating these to leucines had no effect on SFN antagonism of PXR, but the Q285L mutation showed higher affinity towards the ligand RU486, lowering the EC50 and raising the absolute inducibility. To determine if increased inducibility would hold if both residues were changed, a double mutant was made, H407L Q285L, that showed a similar pattern of increased sensitivity to RU486 but also had a much higher basal level of activity, and greater absolute induction by RIF and SR12813. Previous studies of PXR suggest that the αAF region is important in binding of the ketoconazol class of PXR ligands. The K252 lysine reside was mutated to a leucine yielding another mutant with increased basal activity and greater inducibility, this time by RU486, rifampin, and SR12813; however this mutation does not affect the ability of SFN to antagonize PXR. In conclusion, the ability of SFN to prevent ligand binding to PXR does not appear to be due to simple covalent binding to cysteine residues in the ligand binding domain, but other mutations have illuminated residues that are important in ligand binding and specificity, and constitutive activity.

769 NONYLPHENOL ACTIVATES THE CONSTITUTIVE ANDROSTANE RECEPTOR AND CAUSES SEXUALLY DIMORPHIC CHANGES IN P450 EXPRESSION.

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The environmental estrogen, nonylphenol (NP), is one of the most commonly found environmental toxicants in Europe and the United States, and is a breakdown product of nonylphenol ethoxylates. Nonylphenol etoxylates are used in a variety of industrial, agricultural, household cleaning, and beauty products. Recently, we demonstrated that NP also activates the pregnane X-receptor (PXR) and constitutive androstane receptor (CAR) (Hernandez, et al., Tox Sci. 98:416-426) and induces Cyp2b. However, CAR's role in regulating sexually dimorphic P450 expression and induction has not been elucidated. Therefore, wild-type and CAR-null male and female mice were treated with honey as a carrier, NP, or the potent CAR-activator TCPOBOP. Mice were euthanized, livers excised, RNA isolated for Q-PCR, and microsomes prepared for Western blotting. Interestingly, CAR in part, regulates the sexually dimorphic expression of several P450s in untreated mice including members of the Cyp2b and Cyp3a subfamily members such as Cyp2b13 and Cyp3a41 as demonstrated by Q-PCR. Western blots also confirmed that CAR in part directly regulates the expression of some sexually dimrophic P450 subfamily members, including Cyp2b and Cyp3a subfamily members. Interestingly, the loss of CAR in males caused an increase in Cyp2b13 and Cyp3a41 expression suggesting that CAR or androgen-inactivated CAR supresses the expression of these P450s. NP and TCPOBOP induced several P450s in a CAR-mediated sexually dimorphic manner including Cyp2b9, Cyp2b10, Cyp2c38 Cyp2c40, Cyp3a11 and Cyp3a41. Western blots confirmed the CAR-mediated regulation of Cyp2b's and Cyp3a's by NP and TCPOBOP, but the Cyp2c subfamily showed no overall changes in protein expression. In summary, CAR regulates the basal and chemically-inducible expression of several xenobiotic metabolizing P450s including those in the Cyp2b, Cyp2c, and Cyp3a subfamilies.

770 TETRAMETHOXYSTILBENE (TMS), A POTENT CYTOCHROME P450 (CYP) 1A1/1B1 INHIBITOR, ENHANCED BENZO(A)PYRENE (BP)-DNA ADDUCT FORMATION IN MCF-7 CELLS.

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Human exposure to carcinogenic polycyclic aromatic hydrocarbons (PAHs) is unavoidable and results in induction of CYPs 1A1 and 1B1, which biotransform PAHs to metabolites that damage DNA. PAH-DNA adduct levels are considered to reflect the balance between activation by CYP1A1 and 1B1, and detoxification by Phase II enzymes. We therefore hypothesized that down regulation of *CYP1A1* and *1B1* may reduce DNA adduct formation, and utilized a potent CYP1A1/1B1 inhibitor, TMS (1.0 or 4.0 μM) in MCF-7 breast cancer cells exposed to BP (1.0 μM). *CYP1A1/1B1* transcription was monitored by real-time PCR (RT-PCR), CYP1A1/1B1 enzyme activity by ethoxyresorufin-O-deethylase (EROD) assay, and BP-DNA adducts by chemiluminescence immunoassay. In cells exposed to BP for 24h, expression of *CYP1A1* and *1B1* were upregulated 168-fold and 4-fold, respectively. Co-incubation (24h) of cells with BP+1.0 μM TMS, and pre-incubation (24h) with 1.0 μM TMS followed by co-incubation (24h) with BP+1.0 μM TMS gave more than 3000-fold and 17-fold increases in *CYP1A1* and *CYP1B1* expression, respectively. However, despite the increase in expression changes, EROD activity was essentially same in all the (above) treatment groups, indicating no change in enzyme activity with 1.0 μM TMS. With 4.0 μM TMS, EROD activity was inhibited in the groups exposed to: BP+TMS co-incubation, and TMS preincubation with BP+TMS co-incubation by 63.7% and 83.7%, respectively. However, BP-DNA adduct formation was 1427±51 adducts/10^8 nucleotides with BP alone, and adduct levels increased 2- to 3-fold in most groups incubated with TMS, even though the EROD activity decreased at 4.0 μM TMS. These results show that the effect of TMS on CYP1A1/1B1 activity is dose dependent, and that residual CYP1A1/1B1 is sufficient for a substantial level of BP-DNA adduct formation.

771 METABOLISM OF INHALED GLUCOCORTICOIDS BY CYTOCHROME P450 3A ENZYMES.

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Asthma is a chronic inflammatory disease of the airways, estimated to affect 20.5 million Americans (6.2 million children under 18 years). Inhaled glucocorticoids are the bastion of asthma treatment, but approximately 30% of all asthmatics are resistant to steroid treatment. Glucocorticoids are efficiently metabolized by members of the cytochrome P450 (P450) 3A family of enzymes in liver and lung tissues. The major goal of this research is to relate glucocorticoid metabolism by the three major P450 3A enzymes to steroid efficacy in asthmatic patients. We studied the metabolism of five therapeutically relevant glucocorticoids using in vitro incubations of each with CYP3A4, CYP3A5, or CYP3A7. Predicted P450 metabolites were identified for each drug using selected ion monitoring HPLC-MS/MS. All previously reported metabolites, as well as several novel products were identified. Additionally, significant differences in metabolic rates among the drugs were elucidated for each P450 3A isoform. We have looked at regulation of P450 3A enzymes by inhaled glucocorticoids in three different cell lines (A549, BEAS-2B, and HepG2) by quantitative real-time PCR (qrt-PCR). The results showed induction of P450 3A5 in A549 cells and potentially important induction of P450 3A7. Understanding metabolic pathways of these drugs and P450 genetic responses could provide valuable insights into mechanisms of steroid resistance and insensitivity in asthma patients and produce more effective treatments and better disease management. Supported by the Primary Children's Foundation through the Pediatric Pharmacology Program, University of Utah.

772 MICE LACKING THE GENES FOR CYTOCHROME P450 (CYP)1A1 OR 1A2 DISPLAY INCREASED LEVELS OF LIPID PEROXIDATION PRODUCTS AND ENHANCED SUSCEPTIBILITY TO HYPEROXIC LUNG INJURY *IN VIVO.*

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Supplemental oxygen therapy is often required for the treatment of infants having pulmonary insufficiency. However, prolonged exposure to hyperoxia may contribute to the development of bronchopulmonary dysplasia (BPD) in premature infants. Recent studies have suggested a role for cytochrome P450 (CYP)1A enzymes in oxygen-mediated lung injury in rodents. In this investigation, we tested the hypothesis that mice lacking the genes for CYP1A1 or 1A2 would display altered susceptibility to hyperoxic injury in vivo, and increased lung damage would be accompanied by augmented levels of lipid peroxidation products (e.g., F2-isoprostanes,

isofurans, and protein adducts of malodialdehyde (MDA) or 4-hydroxynonenal (4-HNE) in liver and lung. Twelve week-old male wild type (WT) (C57BL/6J) mice or mice lacking the genes for CYP1A1 or 1A2 were exposed to hyperoxia (greater than 95% O2) or room air for 24-72 h. The CYP1A1(-/-) as well as CYP1A2-(-/-) mice were more susceptible to lung damage than similarly exposed WT animals, with 1A2-null mice being the most sensitive. Seventy two hours of hyperoxia caused significant (1.5-4-fold) increases in F2-isoprostanes and isofurans, as measured by GC/MS, in liver and lung of WT, 1A1 (-/-), and 1A2 (-/-) mice, with 1A2-null animals showing the highest levels, followed by 1A1-null animals. Tissue levels of MDA and 4-HNE adducts, as determined by ELISA, were also significantly elevated in hyperoxic animals, with 1A2-null mice displaying the highest levels. The positive correlation between lipid peroxidation products and extent of lung injury supports the hypothesis that lipid peroxidation products significantly contribute to hyperoxic lung injury, and that CYP1A enzymes protect against lung injury by catalyzing the detoxication of lipid hydroperoxides. Further studies could lead to the development of novel strategies for the prevention/treatment of BPD in infants. (Supported by RO1 HL070921 and HL087174.)

773 CYTOCHROME P4501A1 AND ARSENIC IN BENZO(A)PYRENE-INDUCED CARCINOGENESIS.

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The environmental pollutants arsenic and benzo(a)pyrene (BaP) are well-known human carcinogens commonly found together in substances such as cigarette smoke. Arsenic is usually addressed as a non-mutagenic co-carcinogen, with the potential to enhance the effects of mutagens such as BaP, although the mechanisms remain unclear. BaP exerts its carcinogenic effects by forming DNA adducts, leading to DNA replication errors and mutations. The detoxification of BaP in the liver is a multi-step process requiring several enzymes, notably cytochrome P4501A1 (CYP1A1). Furthermore BaP is a potent inducer of transcription of the CYP1A1 gene. Interestingly, depending upon the pathway of action by CYP1A1, BaP may undergo chemical activation into its ultimate carcinogenic metabolite, benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide (BPDE). In this study we examined the impact of arsenic on CYP1A1 activity during BaP exposure, utilizing zebrafish (Danio rerio) as a model species. Co-exposure of zebrafish to arsenic and BaP led to decreased CYP1A1 activity when compared to BaP alone, as measured by the EROD assay. The effect is likely transcriptional, as quantitative PCR detection indicated a decrease of CYP1A1 transcript in zebrafish exposed to both arsenic and BaP when compared to BaP alone. This observed impact of arsenic on CYP1A1 activity may alter cellular levels of BPDE, thus potentially influencing BaP-induced carcinogenesis.

774 BIOLUMINESCENT ASSAYS FOR *IN VITRO* ADMET STUDIES.

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ADMET targets were coupled to firefly luciferase in assays that used the convenience of light output as a readout. In a cell-based approach test compounds were tested for cytotoxicity, glutathione depletion, P450 transcriptional induction and induction of P450 enzyme activity. Inductions of P450 enzyme activities with selective luminogenic substrates were measured and observed as markers for identifying ligands for PXR, CAR, glucocorticoid, aryl hydrocarbon or PPAR-alpha nuclear receptors. Cell-free membrane assays with luminogenic substrates were used to measure P450 or monoamine oxidase enzyme activities and their inhibition by test compounds. IC50s from these bioluminescent assays correlated well with conventional methods in terms of rank order and absolute potency. Each bioluminescent assay relied on the light generating reaction of firefly luciferase, coupling light output with target activity. The assays were insensitive to interference from fluorescent analytes and had low intrinsic background signals giving them high levels of sensitivity and large dynamic ranges for robust, high throughput applications.

775 FICZ, A SUGGESTED NATURAL AHR LIGAND AND A SUPER SUBSTRATE FOR HUMAN CYP1 ENZYMES.

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Several studies point to the fact that light can induce expression of Ah receptor (AhR) regulated genes and that this induction is tryptophan dependent. Among the tryptophan photoproducts identified FICZ binds to AhR with the highest affinity. FICZ is formed when tryptophan in solution is exposed to light (visible and UV) and in human keratinocytes after UVB exposure. FICZ activation of AhR and the subsequent CYP1A1 induction is rapid and transient, due to its efficient metabolism. To evaluate the role of individual phase I and phase II enzymes, detailed studies were carried out using heterologously expressed human CYPs and sulfotransferases (SULTs). CYP1A1, 1A2 and 1B1 generate both mono- and dihydroxylated FICZ metabolites although with different relative amounts. FICZ is an extremely good substrate for all CYP1 enzymes, giving very high specificity constants (Kcat/KM); 4850, 412 and 826 for CYP1A1, 1A2 and 1B1, respectively. These values are 5-50 times higher compared to the values resulted from the same enzyme preparations using the standard substrates ethoxyresorufin and methoxyresorufin. The mono- and di-hydroxylated metabolites are in turn excellent substrates for several human SULTs (SULT1A1, 1A2, 1E1, 1B1). Our data also indicate a strong relationship between the position of the OH-group(s) and the ability to form conjugates. The resulting KM values for 2- and 8-OH-FICZ are between 0.1 and 1.0 μM for SULT1A1 and 1A2. In addition to hydroxylation by CYP1 enzymes, the formyl group is oxidized by aldehyde oxidase to the carboxylic acid metabolite indolo[3,2-b]carbazole-6-carboxylic acid. Recent LC/MS/MS analyses reveal the presence of several FICZ-derived sulfate conjugates in human urine. This latter finding together with the phase I and II kinetics further support the suggestion of FICZ being a natural/endogenous ligand for AhR.

776 DOCKING STUDIES OF A FAMILY OF METHOXYFLAVONES.

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Cytochrome P450 1A2 is involved in the metabolism of a number of important drugs and environmental chemicals such as caffeine, acetaminophen, cigarette smoke components, and estradiol. This enzyme is also one of the most active P450 enzymes in carcinogenesis. Flavonoids, which are phytochemicals abundant in nature and human diet, often interact with P450 1A2 as substrates or reversible inhibitors. We have recently published the synthesis, crystal structures, and preliminary in vitro P450 1A2 inhibition results for a family of methoxyflavones, 2'-, 3'-, and 4'-methoxyflavones as well as 3',4'-dimethoxyflavone. These compounds are very similar in structure to the natural flavone substrates of this enzyme. Some of the compounds synthesized are also isomeric and the differences in their inhibitory activities are useful in the determination of structural features needed for optimum irreversible inhibition. Docking studies have shown that these methoxyflavone molecules dock into a narrow region of the P450 1A2 active site pocket. The planes of the molecules are situated parallel to the heme plane with the larger ring of the flavone oriented toward the heme Fe. The active site pocket seems to be able to accommodate the methoxy group in different docking poses.
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777 WHOLE ZEBRAFISH CYTOCHROME P450 MICROPLATE ASSAYS FOR ASSESSING DRUG METABOLISM AND DRUG SAFETY.

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Cytochrome P450 (CYP) enzymes, particularly CYP3A4 and CYP2D6 catalyze the majority of known drug-metabolizing reactions and many clinically relevant drug-drug interactions are associated with inhibition and/or induction of a specific CYP enzyme. Because of their genetic and physiological similarity to humans, zebrafish show promise as an efficient, predictive animal model for assessing drug metabolism and drug safety. In an earlier study, we have demonstrated that zebrafish exhibit dose-responsive drug toxicity comparable to effects in mouse and human. Several zebrafish CYP genes with high homology to humans or showing a similar catalyzing reaction with mammals, including CYP3A65, CYP1A1, A19, B19, 2K6, 3C1, 2J1 and CYP26D1 have been cloned and characterized. A CYP3A orthologue designated CYP3A65 has been shown homologous to the human CYP3A subfamily. Recently, we have successfully developed microplate-based whole zebrafish CYP3A4 and CYP2D6 functional activity assays for assessing drug metabolism and drug safety. These microplate-based CYP assays use human CYP specific substrates and show high sensitivity and specificity. We found that CYP3A4 functional activity was upregulated in zebrafish treated with 2, 4 -dichlorophenoxyacetic acid (2, 4-D), rifampicin, and dexamethasone, similar to the response of CYP3A4 in humans. Our results were also consistent with a recent report showing that low doses of dexamethasone (10 μM) enhanced the transcription of zebrafish CYP3A65; whereas high doses of dexamethasone (100 μM) did not have such an effect, as measured by whole mount in situ hybridization. Zebrafish CYP2D6 functional activity was upregulated by dexamethasone, but downregulated by 2, 4-D and ethanol. These findings underscore the high degree of CYP conservation in zebrafish. Our whole zebrafish CYP assays provide sensitive and robust tools for moderate throughput

CYP screening in vivo in drug discovery and development. Other microplate-based whole zebrafish CYP functional activity assays including CYP1A2, 2C9, and 2C19 are in development.

778 CHARACTERIZATION OF DRUG-METABOLIZING ENZYMES (DME) AND RECEPTORS IN THE HEPATOMA CELL LINES HEPG2 AND H4IIE, AND PRIMARY RAT HEPATOCYTES.

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In drug development and environmental toxicology, the prediction of induction of drug metabolism is of outstanding interest. In particular, pregnane X receptor (PXR)-mediated induction can be analyzed in vitro in various cell types of rodent or human origin. In our study, human HepG2 and rat H4IIE hepatoma cells as well as primary rat hepatocytes were treated with the prototype inducers/PXR agonists rifampicin and dexamethasone. After 48 hours, RNA was isolated and cDNA synthesised. With TaqMan® Low Density Array cards allowing 384 simultaneous real-time PCR reactions, we analyzed gene expression of 45 phase I and II drug metabolizing enzymes as well as transporters and receptors. In HepG2 cells e.g. 12 of 45 drug metabolism-related proteins like AhR, ABCC2, CYP2B6, CYP3A5/7 and UGT1A1/3-10 were induced by dexamethasone and/or rifampicin. Genes like ABCB11 and CYP4A11/12 could not be detected, and others like ABCB1, CYP2A6, CYP2A1, NR1I2 were endogenously expressed but did not show any response to the treatment. Overall, TaqMan® Low Density Array cards are a fast and customizable method to characterize cells with respect to induction of drug metabolism.

779 ARYL HYDROCARBON RECEPTOR (AHR)-DEPENDENT INDUCTION OF FLAVIN-CONTAINING MONOOXYGENASES.

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Flavin-containing monooxygenases (Fmo) are a family of xenobiotic-metabolizing enzymes that play important roles in chemical detoxification and have been considered not to be inducible. Recent microarray studies in our laboratory showed induction of Fmo2 & Fmo3 by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in liver of C57BL/6J mice with wildtype AHR but not in Ahr-null mice. Fmos were not significantly induced by TCDD in rat liver. The aim of the present study was to further investigated induction of Fmos in mice exposed to different concentrations of TCDD at various times using real time PCR and chromatin immunoprecipitation (ChIP) assays. Time-course experiments showed significant induction of Fmo3 after 2h of exposure, whereas induction of both Fmo2 and Fmo3 were observed following 4h of exposure to 30 μg/kg TCDD. Sex differences in Fmo3 expression were observed for adult mice where high constitutive expression in female livers cannot be further elevated by TCDD while in male adult mice livers TCDD increased the amount of Fmo3 by 6 fold. To investigate the specie differences in TCDD-dependent Fmo induction that exists between mouse and rat, transgenic mice expressing WT rat AHR were analyzed following TCDD exposure. Fmo2 & Fmo3 mRNAs were induced by TCDD in livers of mice carrying the transgenic rat AHR, indicating that the lack of Fmo induction observed in rats is not due to the inability of rat AHR to stimulate Fmo transcription but likely resides in differences between the Fmo gene structures or in the differences in expression of other factors between rat and mouse. ChIP assays from male and female mouse livers showed recruitment of AHR and Arnt to Fmo3 regulatory regions following 2 and 4 h exposure to TCDD indicating that the AHR might take part in the regulation of the Fmo3 gene.

780 STRUCTURAL AND FUNCTIONAL ANALYSIS OF THE RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) FMO GENE.

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Using an NADPH cofactor and FAD prosthetic group, Flavin-containing mono oxygenases (FMO) catalyze the oxygenation of nucleophilic nitrogen, sulphur, phosphorous and selenium atoms in a range of structurally diverse compounds. In many animal systems, individual FMOs are expressed in cell or tissue specific manners, suggesting a finely regulated transcription of FMO genes. However, mechanisms of transcriptional regulation are poorly understood. To better understand FMO gene regulation, we isolated the structural gene encoding a FMO from rainbow trout (*Oncorhynchus mykiss*), using the GenomeWalker™ method. The FMO gene consists of ten exons and the deduced amino acid sequence is 74% identical to FMO1 from Japanese pufferfish(*Takifugu rubripes*), and 52% identical to Zebrafish, and Green puffer (*Tetraodon nigroviridis*) FMO5 amino acid sequences. Six promoters were isolated and shown to span approximately 100 to 3000 bps. The structure of the entire FMO gene, the nucleotide sequence of its 5' flanking region, and the transcriptional initiation sites were determined. Promoter analysis indicated a major transcription initiation site 172 bp upstream from a putative initiation codon. The promoter region of this *FMO* gene also contains potential Osmoregulatory Response Elements (ORE), Ying Yang 1 (YY1) sites, a putative Glucocorticoid Response Element (GRE) half-site, and a CCAAT box. Since other FMO from fish have fewer exons (*Danio rerio*; 7 exon and *Takifugu rubripes*; 8 exon). Intron loss and gain in the evolution of the FMO genes suggest that the basic structural organization of *FMO* genes and the regulatory mechanisms of expression may be species specific (USDA-NRI 2005-35107-16189).

781 ANALYSIS OF A PUTATIVE FOXA1 BINDING SITE IN THE DANIO RERIO CYP1A1 PROMOTER/ENHANCER REGION.

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The CYP1A1 promoter/enhancer region has been invaluable in understanding binding of the aryl hydrocarbon receptor (AHR) with DNA and the recruitment of co-activators. The CYP1A1 gene from zebrafish contains 8 putative core xenobiotic response elements (XREs) organized in two distinct clusters spanning -2608 to -187. Current studies were designed to investigate the role of other transcription factors that may bind the zebrafish CYP1A1 enhancer. In silico analyses identified several putative binding sites within the enhancer region that were also identified in the mouse CYP1A1 enhancer. Electrophoretic mobility shift assays (EMSA) using oligonucleotides corresponding to a FoxA1 (HNF-3)-like site located at position -2548 of the zebrafish CYP1A promoter and Hepa1c1c7 or HepG2 nuclear extracts confirmed binding at this site in vitro. Mutation of the sequence resulted in contradictory effects depending on the construct used in subsequent Luciferase assays. The -2608/-2100Ur construct, in which the zfCYP1A region -2608 to -2100 was ligated immediately upstream of the -580 to +71 promoter region, resulted in ablation of basal and inducible activity while the full length zfCYP1A -2699/+71 construct yielded a 2-3 fold increase in basal and inducible activity. Interestingly, mutation of the putative FoxA1 site located at position -1206 of the mouse CYP1A1 promoter resulted in a modest but statistically significant reduction of TCDD-induced Luciferase levels but had no effect on the magnitude of basal levels. To further address these results, cells were treated with the histone deacetylase I inhibitor, Trichostatin A and the reporter constructs evaluated in the presence of TCDD. In addition, luciferase assays were performed and endogenous CYP1A1 levels were determined by Western blotting in cells treated with siRNA against FoxA1 (HNF-3α). These results suggest that the regulatory mechanism of the protein binding at the FoxA1 site may involve the organization or reorganization of the nucleosome.

782 CHARACTERIZATION OF TCDD-INDUCED HISTONE MODIFICATIONS TO STUDY THE DIFFERENTIAL REGULATION OF THE HUMAN CYP1A1 AND CYP1B1 GENES.

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The Aryl Hydrocarbon Receptor/ARNT heterodimer mediates carcinogenesis by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD or dioxin) and certain polycyclic aromatic hydrocarbons (PAHs) by activating gene transcription. Metabolism of PAHs by CYP1A1 and CYP1B1 plays a major role in carcinogenesis mediated by these compounds. The overall goal of this study is to understand the mechanisms involved in differential regulation of the human CYP1A1 and CYP1B1 genes. We are studying the regulation of these genes in three different human cell lines (MCF-7, MDA-MB 231, and HepG2) as the endogenous expression and induction levels of these genes upon treatment with TCDD differ significantly between these cell lines. To elucidate the mechanisms involved in the differential regulation of these genes, we studied the recruitment of AHR and RNA PolII recruitment at the enhancer and promoter respectively. The data indicated that RNA PolII, but not AHR recruitment, correlates with CYP1A1 and CYP1B1 expression. Subsequently we characterized the different dioxin- inducible chromatin modifications (i.e acetylation, methylation, and phosphorylation) that occur across the CYP1A1 and CYP1B1 enhancer and promoter regions using the Chromatin Immunoprecipi-

tation (ChIP) assay. In accordance with our hypothesis, the results showed H3K9Ac, H3K14Ac and H3K4me3 chromatin modifications at the CYP1A1 and CYP1B1 promoter regions strongly correlate with the TCDD-induction of the CYP1A1 and CYP1B1 genes. These modifications, at least in part are likely to contribute to the differential induction of these genes upon treatment with TCDD. We hypothesize that different transcription factors and coactivators are recruited to the CYP1A1 and CYP1B1 enhancer and promoter regions leading to differential covalent histone modifications.

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783 SUSCEPTIBILITY DIFFERENCES OF THE KOREN PEARS (PYRUS PYRIFOLIA VAR.) INDUCED ALCOHOL DETOXIFICATION IN ALDH2 KNOCKOUT MICE.

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ALDH (aldehyde dehyrogenase) activity has been focused for susceptibility to alcohol or aldehyde toxicity. In addition, Aldh2 knockout mice have been developed by Kawamoto et al. (2003) from C57BL/6 mice. This study was performed in order to clarify functional roles or mechanisms of the Korean pears, a candidate medicinal food for detoxification of alcohol or aldehyde toxicity, with the Aldh2 knockout mice.

In detail, we used male Aldh2-/- mice (n=15) and Aldh2+/+ mice (n=20) (age, 10 weeks-old). Four or five mice per a cage were fasted overnight without wood chip bedding but were allowed to free access of water in the cage before experiment. The animals were administrated with water or juice of the Korean pears by gavages at 200 ul/20g bw. After 30 minutes from the administration, 20% of ethanol in water was given to them by gavages at final dose, 300ul/20g bw. At four hours from the ethanol treatment, they were anesthetized (kethamine 0.4ml, xylazine 0.2ml, saline 6ml), then, we collected blood from their right atrium with cold syringe and rapidly cooled blood in ice. Ethanol and acetaldehyde levels of the blood samples were analyzed by Isse et al's method (2002).

As results, levels of alcohol and acetaldehyde were somewhat reduced by the pear treatment in all mice blood specimens (p=0.04; p=0.36). The levels of acetaldehyde were not significantly reduced by the pear treatment in Aldh2 -/- mice, compared to those in the Aldh2+/+ mice. However, alcohol levels in the Aldh2 -/- mice were somewhat reduced by the pear treatment compared to them in the Aldh2 +/+mice (p=0.08). Therefore, we found that the Korean pears effectively worked for alcohol absorption rather than aldehyde detoxification, and furthermore, Aldh2 -/- mice were more susceptible for the pear-desirable effects against alcohol toxicity, although these results are based on small number of animals.

784 MECHANISMS REGULATING LUNG-SELECTIVE BASAL AND INDUCED CYTOCHROME P450 2S1 EXPRESSION.

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Cytochrome P450 2S1 exhibits a unique expression profile. It is one of the few P450 metabolic enzymes that display both selective expression in extrahepatic tissues and constitutive expression throughout development. Elevated CYP2S1 expression is observed in the hyperproliferative disorders psoriasis and cancer. Increased CYP2S1 expression in colorectal cancer and ovarian cancer correlates with poor prognosis and metastasis, respectively. These data suggest an important role for CYP2S1-mediated metabolism in extrahepatic cells. An important goal of this research is to define the mechanisms underlying the differences in CYP2S1 expression observed between lung and liver cells. Tissue-selective differences are prevalent in CYP2S1 basal expression as well as regulation by the morphogen, all-trans retinoic acid (RA). Our data suggests that these differences are mediated, in part, via transcriptional mechanisms. CYP2S1 promoter luciferase assay was employed to determine important cis-elements within the CYP2S1 promoter required for tissue-selective regulation. Results from these studies indicated that lung A549 cells supported a robust (125-fold) enhancement of basal reporter gene activity and RA significantly induced both reporter luciferase activity and CYP2S1 transcription. The human liver HepG2 cells only moderately (25-fold) supported basal luciferase activity and RA did not induce the promoter-driven reporter gene. A tissue-specific enhancement in basal promoter activity was observed between -227 and -336. This region contains multiple Sp1 sites and a sequence highly similar to the previously characterized Lung-Selective Factor (LSF) binding site observed in CYP2F1. Site-directed mutagenesis and transcription factor/DNA binding assays suggest that both Sp1 and the LSF-like sequences are important for lung-selective expression of CYP2S1. This research was supported by NIH grant #HL60143; NRSA Postdoctoral Fellowship (#F32HL087636) from the National Heart, Lung, and Blood Institute; and the Colgate-Palmolive Postdoctoral Fellowship in In Vitro Toxicology through the Society of Toxicology.

785 CURRENT LEVELS OF POLYBROMINATED DIPHENYL ETHER (PBDE) FLAME RETARDANTS IN THE USA: HUMANS, FOOD, ENVIRONMENTAL SAMPLES AND DAILY INTAKE.

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PBDEs are widely used flame retardants worldwide. Levels in US nursing mothers' milk are 10-100 times higher than in Europeans. US food PBDE levels are somewhat higher than European levels. US fish have highest PBDE levels, median 616 pg/g, meat, median 190 pg/g and dairy median 32 pg/g. US dietary intake is highest from meat, then dairy and fish. Household dust levels are 705-69283 ng/g. 1973 blood shows higher dioxins and PCBs but lower PBDEs than 2003 blood. Household dryer lint is lower in PBDE content than household dust. Older computer and computer monitor casings are high in PBDEs, from 77-1536 ng/cm2 in the USA. Food of animal origin may provide substantial amounts of PBDEs and dust may also contribute significantly. Vegans have lower levels of blood PBDEs and vegetables have lower levels than animal food. Cooking can lower the amount of fat and PBDEs. Fast food contains PBDEs, from 3417-23381 pg/per serving. Average total intake of PBDEs in adults is 75 ng/day, 81 ng/day in children and 2265 ng/day in nursing infants. Partitioning between blood and milk suggests larger congeners such as the fully brominated BDE, 209, although frequently present, have more difficulty moving from blood to milk. Human urine to date does not show any measurable PBDEs. Workers at some electrical recycling facilities are exposed to high levels in air. We estimate their PBDE intake to be 2763 ng/day compared to the general population intake of 75 ng/day. Air is a minor contributor to body burden but in occupational exposures air may play a larger role. Indoor air is usually higher in PBDEs than outdoor air. At this time there is no way to compare toxicity between PBDE congeners as there is with dioxins. PBDEs have been found in animal studies to be associated with reproductive and developmental pathology, endocrine disruption, brain damage and possibly, at high doses, cancer.

786 OCTYLPHENOL AND NONYLPHENOL-INDUCED APOPTOSIS IN MOTOR NEURON IS RELATED TO FAS-FAS LIGAND PATHWAY.

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Octylphenol (OP) and nonylphenol (NP) are common environmental contaminants, known to disrupt the reproductive and endocrine system. OP and NP were been reported to have estrogenic activity and shown to have potential reproductive toxicity. However, their's influence on motor neuron function remains unclear. In this study, we used mouse motor neuron derived cells (MN-1) and mouse neuron derived cells (SN56) to investigate the potential toxicity of OP and NP. Our results showed that the cytotoxic effects of OP and NP involved DNA fragmentation, the major characteristic of apoptosis. The OP and NP-induced apoptosis was concomitant with the increased activity of Caspase-8 and -3. In addition, both Fas and Fas ligand (FasL) protein expressions were markedly increased in the OP and NP-exposed MN-1 cells. These results suggested that OP and NP are able to trigger apoptosis in MN-1 cells via a pathway dependent on caspase activation and Fas-FasL interaction. In particular, SN56 cell had a lower sensitivity to the toxicants than MN-1 cells, thereby suggesting that the toxic stress response may differ depending on the neuron cell type. These findings offer new perspectives for understanding the fundamental mechanisms in endocrine disruptors-induced apoptosis in motor neuron derived cells.

787 TRIBUTYLTIN INDUCED APOPTOSIS IN RAT LEYDIG CELL LINE, R2C, THROUGH THE FAS/FAS L PATHWAY.

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Tributyltin chloride (TBT), an endocrine-disrupting chemical, has been used as a heat stabilizer, agricultural pesticide, and component of antifouling paints. TBT has been reported to have estrogenic activity and shown to have potential repro-

ductive toxicity. However, its influence on reproductive system function remains unclear. This study investigated the mechanisms involved in induction of apoptosis by TBT in rat leydig cell line, R2C. To determine whether Fas/Fas ligand (FasL) pathway involved by TBT, Fas and FasL proteins and caspase-3, -8 activities were tested in R2C cells by western blotting and ELISA. The results showed that TBT decreased the cellularity; induced apoptotic death and enhanced the expression of Fas and FasL mRNA as well as proteins in R2C cells. In addition, TBT also increased caspase-3 and -8 activities in a does dependant manner, whereas specific caspase inhibitors (caspase-3, 8) suppressed TBT-mediated cell death. These findings suggest that TBT may induce apoptosis by altering the expression of Fas and FasL in R2C cells so as to affect the reproductive system function.

788 O, P'-DDT INDUCE MAST CELL DEGRANULATION AND ENHANCE IGE-MEDIATED RELEASE OF ALLERGIC MEDIATORS.

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Dichlorodiphenyltrichloroethane (DDT) has been used as an insecticide and to prevent devastating malaria in many people in the tropical zones. Although o,p'-DDT has been shown to be xenoestrogenic effect, the involvement of allergic effects is not understood. In this study, we investigated the effects of o,p'-DDT on the ability to modulate the release of allergic mediators from mast cells. o,p'-DDT induced the beta-hexosaminidase and histamine release in splenocytes and mast cells co-culture system. o,p'-DDT induced IgE production in dose dependent manner. In addition to, o,p'-DDT increased IL-4 production and gene expression. These findings suggested that o,p'-DDT might promote allergic diseases by inducing and enhancing mast cell degranulation.

789 STRUCTURE-ACTIVITY RELATIONSHIPS FOR PEFLUOROCARBON-MEDIATED TRANSCRIPTIONAL RESPONSE IN RAT AND HUMAN HEPATOCYTES.

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The perfluorinated alkyl carboxylates and sulfonates (PFAAs) are of emerging concern because of the environmental penetration and persistence of the 8-carbon analogs, perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS). Manufacturers have explored alternative formulations with comparable performance characterostics but substantially lower biopersistence. In some applications, lesser carbon number PFAAs may offer these properties. Safety assessment of these smaller PFAAs can be explored by comparing their biological activities to those induced by the extensively studied 8-carbon analogs, PFOA and PFOS. The purpose of this investigation was to explore structure-activity correlations for PFAA-induced expression of acyl CoA oxidase (ACoA) and Cyp4A1 transcripts, which have been previously identified as specific and sensitive bioindicators of the response to PFOA and PFOS in vivo. Rat and human primary hepatocytes were exposed in culture to selected PFAAs over a suitable concentration range, after which the cells were harvested, the nucleic acids extracted and transcripts quantified by real-time quantitative PCR. Both PFOA and PFOS caused a dose-related increase in expression of ACoA and Cyp4A1 in rat hepatocytes, with peak responses at 25 μM – 50 μM; higher concentrations caused morphological changes indicative of cell injury, particularly for PFOS. A similar dose-dependence was observed for primary human hepatocytes, but the degree of stimulation was substantially less and of questionable significance. Within the PFAAs, induction of ACoA and Cyp4A1 transcripts in primary rat hepatocytes increased with increasing carbon number and was much greater for PFOA than PFOS; The transcriptional response to PFBA was approximately 1/4th that for PFOA. These results indicate that transcriptional responsiveness of isolated primary hepatocytes exposed in culture increases with increasing carbon number and that rats are substantially more responsive to PFAAs than humans.

790 GLUTATHIONE LEVELS MODULATE THE NEUROTOXICITY OF POLYBROMINATED MIXTURE DE-71 IN MOUSE NEURONS.

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Polybrominated diphenyl ether (PBDE) flame retardants have become widespread environmental contaminants. Exposure is believed to occur primarily through diet and dust. Infants and toddler have highest exposure through maternal breast milk and household dust. The primary concern for adverse health effects of PBDEs relates to their potential developmental neurotoxicity, which has been found in a number of animal studies. Information on the possible mechanisms of PBDE neurotoxicity is limited, though some studies have suggested that PBDEs may elicit oxidative stress. The present study examined the in vitro neurotoxicity of DE-71, a pentaBDE mixture, in primary neurons obtained from wild-type and Gclm knockout mice, which lack the modifier subunit of glutamate-cysteine ligase and, as a consequence, have very low levels of glutathione (GSH). Cerebellar granule neurons and hippocampal neurons from Gclm (-/-) mice displayed high sensitivity to DE-71 toxicity, which was antagonized by antioxidants melatonin (200 uM) and the membrane permeable GSH delivery agent GSH ethylester (2.5 mM). When CGNs from Gclm (+/+) mice were exposed to the GSH synthase inhibitor buthionine sulfoximine (BSO; 25 uM for 24 h), the toxicity of DE-71 was significantly increased, and Gclm (+/+) CGNs were as sensitive as CGNs from Gclm (-/-) mice not treated with BSO.
DE-71 also caused a time-dependent increase in the levels of reactive oxygen species, which was more pronounced in CGNs from Gclm (-/-) mice. The nature of DE-71-induced cell death was also investigated. DE-71 caused a concentration- dependent apoptotic cell death. These findings suggest that the developmental neurotoxicity of PBDE may involve oxidative stress, and that individual with genetic polymorphism leading to lower GSH levels may be more susceptible to their adverse effects. Supported in part by a grant from NIH (P30ES07033).

791 LUNG MORPHOLOGY AND RESPIRATORY MECHANICS ARE SIGNIFICANTLY ALTERED IN RATS GESTATIONALLY EXPOSED TO 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN.

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In mature laboratory animals, adverse effects on the lung have been observed after chronic exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), including alveolar-bronchiolar metaplasia, hyperplasia of the bronchiolar epithelium, adenomatous hyperplasia and keratinizing squamous cell carcinoma. We hypothesized that the developing lung may also be adversely affected by TCDD which may represent a significant factor contributing to the high rate of perinatal mortality observed after gestational exposure. Pregnant Holtzman rats received a single dose of TCDD (1.5 or 6μg/kg, po) on gestation day (GD) 10 or a vehicle control with fetal and neonatal analysis occurring on GD20 or post natal day (PND) 7. High rates of perinatal mortality were observed on GD20 (~20% and ~40%) and PND7 (~40% and 100%) in the TCDD exposure groups (1.5 and 6 μg/kg TCDD, respectively). Pups gestationally exposed to TCDD exhibited signs of lung hypoplasia indicated by significant decreases in normalized wet and dry lung weights. Morphometric analysis of GD20 and PND7 fixed lung tissue sections revealed that treated pups had significant decreases in total airspace area while having significantly wider septa separating the airspaces when compared to controls. Assessment of respiratory mechanics on PND7 pups revealed functionally different pressure-volume curves in TCDD exposed pups when compared to control animals. Mathematically modeling the pressure-volume curves generated lung compliance and hysteresis values that were both decreased in the TCDD exposed animals. Together, these results identify the developing lung as target for the toxicity of TCDD and sheds light on the observation that lung hypoplasia is accompanied by significant alterations in morphology and respiratory mechanics.

792 XENOBIOTIC GEOMETRY AND MEDIA PH DETERMINE CYTOTOXICITY THROUGH SOLUBILITY.

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Biological activity is dependent upon the solubility of the xenobiotic, but very little is known about the factors that influence solubility in cell culture. In the present study we used a series of monofluorinated analogues (F-PCBs 3) of 4-chlorobiphenyl (PCB 3) as model compounds to investigated the effects of the dihydral angle, modeled with semiempirical and ab initio molecular orbital methods, and the dipole moment, calculated in vacuum and in water, on solubility, measured by diffraction spectroscopy. As biological endpoint we determined cytotoxicity in human liver (HepG2), colon (CaCo2) and skin keratinocyte (HaCaT) cell lines. We found a strong positive correlation between the dihedral angle, the cavitation energy, the solubility, and the cytotoxicity in those three human cell lines. The dipole moment was of minor influence. We also analyzed the influence of pH changes in cell culture medium containing 10% fetal bovine serum (FBS), changes that may occur in many cell culture media by temporary exposure to non-CO2–buffered air. We observed that the solubility was strongly affected by the pH

and that this effect was not reversed by subsequent pH re-adjustment to neutral levels. In addition, the cytotoxicity study showed that both, the actual pH and the pH history of a medium containing FBS, had a strong influence on toxicity. We hypothesize that pH-driven changes in the tertiary and quaternary structure of albumin are responsible. These observations have implications for studies of the biological activity of semi-soluble compounds, like PCBs and related compounds. (Supported by P42ES013661 from NIEHS, Alexander von Humboldt Foundation, Germany, and DAMD 17-02-1-0241 from DOD).

793 DISPARITY IN THE INDUCTION OF OXIDATIVE DNA DAMAGE AND REPAIR BY POLYCHLORINATED BIPHENYLS (PCBs) IN HUMAN MCF-7 AND MDA-MB-231 BREAST CANCER CELLS.

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The purpose of this research is to examine the differences in the induction of oxidative stress, DNA damage and repair, and apoptosis by polychlorinated biphenyls (PCBs), including PCB52 and PCB77 in human MDA-MB-231 and MCF-7 breast cancer cells. Results from Western blot analyses confirmed that treatment of cells with PCBs resulted in significant increases in protein expression of p53, caspase 8, and caspase 9. This evidence suggests the induction of caspase-dependent apoptosis by PCB52 and PCB77 in both human breast cancer cells. The data also showed that PCB52 and PCB77 induced increases in intracellular levels of reactive oxygen species (ROS) and decreases in intracellular glutathione in MDA-MB-231 and MCF-7 cells. Additionally, at non-cytotoxic concentrations both PCB52 and PCB77 induced decreases in intracellular NAD(P)H and NAD^+ in MDA-MB-231 cells but not in MCF-7 cells. We noticed that PCB-induced decreases in intracellular NAD(P)H in MDA-MB-231 cells was blocked by three types of poly(ADP-ribose)polymerase-1 (PARP-1) inhibitors. The catalytic activation of PARP-1 in cells treated with PCB52 and PCB77 was confirmed by detection of the presence of polymers of ADP-ribose-modified PARP-1 using Western blotting. Further investigation verified that both PCB52 and PCB77 induced increases in the number of DNA strand breaks in MDA-MB-231 cells but not in MCF-7 cells as measured by the single-cell gel electrophoresis (Comet) assay. These results suggest that decreases in intracellular NAD(P)H in PCB-treated MDA-MB-231 cells are primarily due to reduction in intracellular NAD^+ pool mediated by PARP-1 activation through formation of DNA strand breaks in MDA-MB-231 cells. Overall, this evidence demonstrates that both PCB52 and PCB77 may induce disparity in the induction of DNA damage and repair in human MCF-7 and MDA-MB-231 breast cancer cells. (This work was supported by the National Science Council, Taiwan, through grant NSC95-2314-B-005-001).

794 ENHANCED POTENCY OF PBDE METABOLITE 6-OH-BDE47 TO AFFECT CA^{2+} HOMEOSTASIS AND EXOCYTOSIS IN PC12 CELLS.

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Evidence of toxicity and environmental abundance of the brominated flame retardants polybrominated diphenyl ethers (PBDEs) is increasing. Hydroxylated metabolites of these persistent organic pollutants have also been detected in wildlife and humans. Increase of intracellular calcium ($[Ca^{2+}]_i$), likely due to release from intracellular calcium stores, appears a common mechanism of action for *ortho*-PCBs and high concentrations of PBDEs.

To investigate whether these metabolites increase the neurotoxic potency of PBDEs, we measured the effects of a hydroxylated metabolite of BDE47 (2,2',4,4'-tetrabromodiphenyl ether; 6-OH-BDE47) on $[Ca^{2+}]_i$ (using the fluorescent dye Fura-2) and neurotransmitter release (by amperometry) in rat pheochromocytoma (PC12) cells.

Previously we have shown that in PC12 cells exposed to the parent compound BDE47, a gradual increase in $[Ca^{2+}]_i$ is observed only at 20 μM. Exposure to 6-OH-BDE47 (≥1 μM) results in a biphasic increase in $[Ca^{2+}]_i$. Shortly after the onset of exposure, a transient increase in $[Ca^{2+}]_i$, which coincides with an increase in exocytosis, is observed. Additionally, a late, concentration-dependent increase is observed. By using thapsigargin and the mitochondrial uncoupler carbonylcyanide-4-(trifluoromethoxy)-phenylhydrazone (FCCP) to selectively deplete endoplasmic and mitochondrial calcium stores, we show that the initial increase originates primarily from the endoplasmic reticulum, whereas the late increase originates primarily from mitochondria.

These results clearly show that hydroxylated metabolite 6-OH-BDE47 is far more potent in disturbing calcium homeostasis and neurotransmitter release than the parent compound BDE47. Additionally, based on the observed and analogue mechanism of action, a cumulative neurotoxic effect of PBDEs and ortho-substituted PCBs can no longer be ruled out.

795 FREE RADICAL FORMATION BY UV IRRADIATION OF DECA-BROMODIPHENYL ETHER (DECA-BDE).

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Among the polybrominated diphenyl ethers (PBDEs), deca-bromodiphenyl ether (deca-BDE) is the most commonly used brominated flame retardant. Levels of PBDEs in the environment and in humans have recently increased. Photodegradation of deca-BDE with UV light results in PBDEs with fewer bromines. We hypothesize that photodegradation will result in free radical formation. Electron paramagnetic resonance (EPR) was used to detect free radical formation using 5,5-dimethyl-1-pyrroline N-oxide (DMPO) and 2-methyl-2-nitrosopropane (MNP) as spin traps. A commercial deca-BDE (DE-83R) and octa-BDE mixture (DE-79), deca-bromobiphenyl (PBB 209), deca-chlorinated biphenyl (PCB 209) and phenyl ether (PE) were dissolved in tetrahydrofuran (THF), dimethylformamide (DMF), or toluene. Samples were irradiated with a xenon lamp with a 309 nm or 280 nm cut-off filter. Irradiation of deca-BDE resulted in EPR spectra that were distinctly different in each solvent; spin adduct concentrations increased with the deca-BDE concentration (THF and DMF) and irradiation time (THF, DMF and toluene). The signals of octa-BDE, PBB 209, PCB 209, and PE were much weaker than deca-BDE and the signal of PBB 209 was stronger than PCB 209, indicating that bromine and an ether bond enhance the radical formation. With deca-BDE in THF, an MNP adduct of hydrogen atom was detected. In deuterated THF, a different set of EPR spectra was observed. These results suggest that the radical formation of deca-BDE is related to hydrogen abstraction from THF. The hyperfine splittings of the spin adducts suggest radical formation is initiated, or enhanced, in organic solvents with deca-BDE and light via debromination and hydrogen abstraction from the solvents. Our findings that UV irradiation of deca-BDE in organic solvents produces oxidizing free radical indicates that synergism in toxicity between PBDEs and UV light should be examined. (Supported by NIEHS P42ES013661, DOD DAMD17-02-1-0241, EPA R-82902102-0 and CHEEC)

796 POLYBROMINATED DIPHENYL ETHERS (PBDES) IN LEACHATES FROM SELECTED LANDFILL SITES IN SOUTH AFRICA.

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The last few decades have seen dramatic growth in the scale of production and the use of(PBDEs as flame retardants. Consequently, PBDEs such as BDE-28,-47,-66,-71,-75,-77,-85,-99,-100,-119,-138,-153,-154 and -183 have been detected in various environmental matrices. Generally, in South Africa once the products containing these chemicals have outlived their usefulness, they are discarded into landfill sites. Consequently,the levels of PBDEs in leachates from landfill sites may give an indication of the general exposure and use of these compounds. The present study was aimed at determining the occurrence and concentrations of common PBDEs in leachates from selected landfill sites.The extraction capacities of the solvents were also tested. Spiked leachate samples were used for the recovery tests. Separation and determination of the PBDEs congeners were carried out with: Gas Chromatograph equipped with Ni63 electron capture detector.The mean percentage recoveries ranged from 63-108% (n=3) for landfill leachate samples with petroleum ether giving the highest percentage extraction. The mean concentrations of PBDEs obtained ranged from ND-2670 pg l-1, ND-6638 pg l-1, ND-7230 pg l-1, 41-4009 pg l-1, 90-9793 pg l-1 for Garankuwa, Hatherly, Kwaggarsrand, Soshanguve and Temba landfill sites respectively. Also BDE-28,-47,-71 and -77 were detected in the leachate samples from all the landfill sites; and all the congeners detected in two of the oldest landfill sites. The peak concentrations were recorded for BDE-47 at three sites,-71 and -75 at two sites. The highest concentration, 9793 ± 1.5 pg l-1, was obtained for Temba landfill site with the highest BOD value. This may suggest some influence of organics on the level of PBDEs. Considering the leaching characteristics of brominated flame retardants, there is a high possibility that with time these compounds may infiltrate into the groundwater around the sites since most of the sites are not adequately lined.

797 EFFECTS OF THE BROMINATED FLAME RETARDANT HBCD ON CA^{2+} HOMEOSTASIS IN PC12 CELLS.

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Brominated flame retardants (BFRs), like polybrominated diphenyl ethers (PBDEs), are ubiquitous environmental pollutants. Due to their persistence and toxic potential, PBDEs might be in part replaced by other, presumably less toxic, BFRs like hexabromocyclododecane (HBCD). Some PBDEs, eg., BDE47, have been shown to disturb Ca^{2+} homeostasis and synaptic plasticity (Dingemans et al. (2007), Env. Health. Persp. 115:865). Consequently, the aim of this study was to investigate whether the commercial HBCD mixture or its three main stereo-isomers (α, β and γ) are able to disturb intracellular Ca^{2+} homeostasis.
The effects of the commercial HBCD mixture on the basal cytosolic Ca^{2+} concentration and frequency of spontaneous Ca^{2+} fluctuations as well as on depolarization-evoked Ca^{2+} influx via voltage-gated Ca^{2+} channels were assessed in PC12 cells using the fluorescent ratio dye Fura-2 AM.
The results demonstrate that exposure to the commercial HBCD mixture has no effect on the frequency of spontaneous Ca^{2+} fluctuations or the basal cytosolic Ca^{2+} concentration. However, HBCD is able to affect the depolarization-evoked Ca^{2+} influx in a dose and time dependent manner at micromolar concentrations (2-20 μM), suggesting that intracellular accumulation of the compound plays a role in the observed effects. Moreover, all three stereo-isomers were able to induce this effect, with the α- and γ- isomer having the highest potency.
Though the responsible mechanisms need to be further unraveled, it can already be concluded that HBCD has neurotoxic potency as it disturbs depolarization-evoked Ca^{2+} influx.

798 PERFLUOROOCTANOIC ACID ALTERS T LYMPHOCYTE PHENOTYPES AND CYTOKINE EXPRESSION IN MICE FOLLOWING 21-DAY ORAL EXPOSURE.

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Perfluorooctanoic acid (PFOA) is a member of perfluorinated compounds used in commercial applications and has been detected in environmental matrices. This study focuses on whether PFOA affects the immune systems in mice. Male ICR mice were exposed continuously to 0, 2, 10, 50, and 250 ppm of PFOA in drinking water for 21 days. Food and water consumption decreased in mice exposed to 250 ppm of PFOA. Mean body weight gain was reduced significantly in mice treated with 50 and 250 ppm of PFOA. The relative weight of spleen and thymus increased in mice exposed to 50 and 250 ppm of PFOA. Exposure to PFOA by drinking water altered T lymphocyte populations in spleen and thymus, differently. In spleen, PFOA ranging from 2 to 250 ppm decreased CD8+ single-positive lymphocyte populations; CD4+ lymphocyte populations were increased in mice treated with 50 and 250 ppm of PFOA. Exposure to 250 ppm of PFOA increased CD8+ lymphocyte populations in thymus, whereas the number of CD4+ lymphocytes was not altered. PFOA altered the expression of pro-inflammatory cytokines (tumor necrosis factor α, interleukin-1β, and interleukin-6), and c-myc in spleen and thymus. Gene expressions of inflammatory cytokines were elevated in spleen of mice treated with the highest dose of PFOA, whereas not in thymus. The expression of c-myc was increased in both spleen and thymus. In conclusion, our data demonstrate that PFOA has immunomodulatory effect by modulating T lymphocyte phenotypes and gene expression of pro-inflammatory cytokines.
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799 IN VITRO HEPATIC METABOLISM OF PBDE CONGENERS 99 AND 209 BY CHINOOK SALMON.

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Polybrominated diphenyl ethers (PBDEs) are a class of brominated flame retardants that persist in the environment and whose residues have been increasing exponentially in humans and wildlife over the past twenty years. One of the primary human exposure routes for PBDEs appears to be consumption of contaminated foods. Of particular concern in the Pacific northwest are the PBDE residues in resident Puget Sound Chinook salmon (*Oncorhynchus tshawytscha*), which are among the highest in salmonids and are dominated by low molecular weight congeners, primarily BDE 47. However, PBDE production and use are dominated by higher brominated PBDEs, suggesting that the congeners detected in Puget Sound salmon may result from *in vivo* debromination of higher molecular weight congeners. We are investigating debromination of PBDEs in Chinook hepatic microsomal fractions *in vitro* under incubation conditions supporting cytochrome P450 (CYP450) and thyroxine deiodinase (DI)-mediated activities. Preliminary studies in our lab indicate an overall inefficient debromination of BDE 209 by Chinook liver microsomes, but an observable dose and time-dependent debromination of BDE 99 by Chinook liver microsomes resulting in conversion to BDE 49 after 1.5 and 24 hour incubation periods. No conversion of BDE 99 to BDE 47 by Chinook liver microsomes has been observed. The results of the study suggest that the high levels of BDE 47 in resident Chinook are likely not a result of enzymatic debromination of the higher molecular weight PBDE congeners such as BDE 99 or 209. Future work will continue to evaluate hepatic metabolism and debromination of PBDEs, with an emphasis on identifying the enzyme pathway(s) involved in PBDE metabolism.
This work was supported by NOAA NA05NS4781253.

800 INVESTIGATION OF 4-CHLOROBIPHENYL (4-CB) AND ITS PHASE I METABOLITES AS SUBSTRATES OF HUMAN RECOMBINANT PROSTAGLANDIN H SYNTHASE-2.

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Polychlorinated biphenyls (PCBs) are environmental pollutants primarily metabolized by hepatic cytochromes P-450 (CYPs) to phenols, catechols and hydroquinones which can undergo redox interconversion to quinones. These quinones are electrophilic and are highly reactive to nucleophiles, such as proteins and DNA. Recent studies have shown that electron rich xenobiotics, such as phenolic compounds, can serve as reducing cosubstrates during the peroxidase reaction of prostaglandin H synthase (PGHS). Therefore, we hypothesize that PGHS oxidizes the dihydroxy metabolites of PCBs to the corresponding quinones. 4-Chlorobiphenyl (4-CB) and its analogues, 4'-hydroxy-4-chlorobiphenyl (4-CB-4'-OH), 2',3'-dihydroxy-4-chlorobiphenyl (4-CB-2',3'-OH), 3',4'-dihydroxy-4-chlorobiphenyl (4-CB-3',4'-OH), and 2',5'-dihydroxy-4-chlorobiphenyl (4-CB-2',5'-OH) were selected as the model compounds to test the hypothesis. The model compounds were initially tested for their ability to inhibit PGHS-2 activity. Instead of inhibition, the dihydroxy 4-CBs increased the formation of prostaglandin F2α. Based on these findings, further investigation using incubation methods of the 4-CB-2',3'-, 3',4'-, 2',5'-OH with human recombinant PGHS-2 (hPGHS-2) were conducted using N-acetyl cysteine (NAC) as a trapping agent for the corresponding electrophilic quinones. We found that hPGHS-2 catalyzed oxidation of 4-CB-2',5'-, 2',3'-, and 3',4'-OH to the corresponding quinones which rapidly reacted with NAC to form mono-, and di-NAC adducts in vitro. These adducts were identified and quantified by LC-MS and 1H NMR. The addition of NAC to quinones occurred via a nucleophilic addition at the β-position of the 4-CB-benzoquinones. PGHS-2 may, therefore, contribute to the activation of PCBs and metabolites, especially in non-hepatic tissues. (Supported by P42 ES 013661)

801 RESIDUAL PAHs, PCBs, PCDDs, AND PCDFs IN SOIL AND HOUSE DUST FOLLOWING AN INDUSTRIAL CHEMICAL RELEASE AND FIRE.

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In May of 2000, a train derailment in Eunice, LA, resulted in the release and combustion of chlorinated solvents. To characterize residual contamination due to products of combustion of these chemicals, soil and dust samples were collected from in and around homes in Eunice and in six surrounding communities. Samples were analyzed for the presence of 16 polycyclic aromatic hydrocarbons (PAHs), 12 polychlorinated biphenyls (PCBs), and 17 polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). Analytical results for Eunice were compared with those for the surrounding communities. Median values were used for measures of central tendency and standard non-parametric statistical procedures were employed. In no case was the median level in Eunice statistically significantly higher than the median for background communities for dust or soil samples for any individual chemical. The ratio of the median level among samples in Eunice to the median level among samples for the background communities was calculated to provide a quantitative measure of the relative concentrations. The overall median of these ratios in Eunice compared to the background communities was 0.86 (95% CI: 0.41-1.40). Combined 2,3,7,8-TCDD Toxicity Equivalents

(TEQs) were calculated for PCBs, PCDDs, and PCDFs using current WHO toxic equivalency factors (TEFs). TEQs in samples from Eunice were not statistically significantly different from that in samples from the background communities. These results provide background concentrations for several important chemicals in residential dust and soil and indicate that the residual impact of combustion products from the fire on this community was negligible.

802 THE EFFECT OF PRENATAL 2, 3, 7, 8-TETRACHLORODIBENZO-*P*-DIOXIN EXPOSURE ON *CYP1A1*, *CYP2E1*, *EGF*, AND *EGFR* EXPRESSION IN ADULT *AHR* CONGENIC MICE.

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The halogenated aromatic hydrocarbon 2,3,7,8–tetrachlorodibenzo-*p*-dioxin (TCDD) is a ubiquitous environmental contaminant known to elicit a wide range of toxic effects. TCDD is thought to cause developmental perturbations primarily through the aryl hydrocarbon receptor (*Ahr*) signaling pathway. Especially at small dosage levels, we know very little about the duration of gene expression changes due to TCDD exposure, and whether any persistent effects are associated with alleles at the *Ahr* locus. We therefore tested whether *Cyp1a1*, *Cyp2e1*, *Egf*, and *Egfr* expression was altered in 10-week-old congenic (Ahr^{b1}/Ahr^{b1} and Ahr^{d}/Ahr^{d}) mice maternally dosed on gestation day 13 with TCDD (0, 0.01, 01, or 1 μg TCDD/kg body weight). We expected to detect some control versus treatment differences in gene expression (specifically with *Cyp1a1* and *Egfr*) in the more 'sensitive' Ahr^{b1}/Ahr^{b1} mice, but little or no difference in the more resistant Ahr^{d}/Ahr^{d} mice. In this study, we quantified gene expression in the liver tissue of each sample with real-time PCR and used litter means in the analysis. The results for *Cyp2e1* expression showed the hypothesized trend: altered (decreased) expression in all treatment groups of the Ahr^{b1}/Ahr^{b1} strain but only at the highest dosage treatment group in the Ahr^{d}/Ahr^{d} strain. For all other endpoints, however, the Ahr^{d}/Ahr^{d} mice showed more of a response to TCDD (increased levels of *Cyp1a1* and *Egfr* in the 0.1 μg/kg group and decreased *Egf* levels in the 1 μg/kg group). We hypothesized that this may have been because of the greater efficiency of the Ahr^{b1}/Ahr^{b1} mice in eliminating TCDD (due to the higher binding affinity of their aryl hydrocarbon receptor). Whatever the reason, our results further suggest that the *Ahr* locus plays an important role in mediating the effects of TCDD and that persistent effects may be detected even from small exposures.

803 *IN VITRO* ANTI-ANDROGENICITY OF PBDES, HBCD, TBP AND AND HYDROXYLATED AND METHOXYLATED PBDES BASED ON A YEAST BIOASSAY.

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Polybrominated diphenyl ethers (PBDEs) have been shown to be susceptible to several metabolic processes. Hydroxy- and methoxylated PBDEs (OH-PBDEs, CH3O-PBDEs) have recently been reported to be present in various biotic samples including wildlife and human. Some in vitro studies have indicated that OH-PBDEs are agonists of the estrogen receptor, thus potential endocrine disruptors.
In this study, androgen receptor (AR) agonist and antagonist potencies of several brominated FR and PBDE metabolites have been studied in a recombinant yeast bioassay that expresses the human androgen receptor (AR) and yeast enhanced green fluorescent protein (γEGFP). 40 compounds were tested at concentrations ranging from 0.01 up to 25 μM. None of the compounds tested exhibited agonistic potencies in the yeast androgen bioassay. However, 19 out of 40 BFRs and PBDE derivatives showed antiandrogenic effects when they were co-exposed with the endogenous AR ligand testosterone (T, 1 μM). Significant AR antagonism was found for BDE19, 47, 49 and TBP, which concentration-dependently decreased the T-induced response with more than 80% and at 25 μM. Flutamide completely decreased T-induced fluorescence with an IC50 value of 1 μM. Exposures to the OH-PBDEs resulted in a concentration-dependent antiandrogenicity in most of the cases, with reduction of T-induced response between 50 and 90% of control at 25 μM. Some of the CH3O-PBDEs also displayed antiandrogenic activity, but not more than 50% of T-induced response, except for 2-CH3O-BDE28 (almost 90% decrease at 25 μM, IC50 value of 8,9 μM). We conclude that some of these BFRs, especially some PBDE metabolites, exhibit antiandrogenic properties. Even in some cases with the same or even higher potency than flutamide, a selective AR antagonist.

804 2, 2', 3, 3', 6, 6'-HEXACHLOROBIPHENYL (PCB 136) ATROPISOMERS BIND ENANTIOSELECTIVELY TO HEPATIC MICROSOMAL CYTOCHROME P450 ENZYMES.

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PCB 136 is a neurotoxic PCB congener that displays axial chirality. (+)-PCB 136 has been shown to be enriched in tissues from mice treated with racemic PCB 136. Spectral interactions of PCB 136 atropisomers with hepatic microsomal cytochrome P450 (CYP) enzymes were investigated to test the hypothesis that enantioselective binding to CYP enzymes causes the enrichment of (+)-PCB 136 in vivo. Female C57Bl/6 mice were treated by intraperitoneal injection with phenobarbital (PB, 400 μmol/kg body wt), dexamethasone (Dex, 123 μmol/kg body wt), β-naphthoflavone (NF, 100 mmol/kg body wt) or corn oil (CO) (10 ml/kg body wt) for three consecutive days. Hepatic microsomes were prepared and binding of PCB 136 atropisomers to microsomal CYP enzymes was assessed by difference spectroscopy using a dual wavelength/split beam spectrometer. Sample and reference microcuvettes both contained microsomes diluted to 4 nmol CYP/ml in 0.1 M phosphate buffer (pH 7.5). The maximal absorbance change (ΔA_{max}) values, a measure of PCB binding to CYP enzymes, were derived by nonlinear regression analysis from the absorbance change versus PCB concentration curves. Binding of racemic PCB 136 was greatest with microsomes from PB- (ΔA_{max}=0.027) followed by Dex- (ΔA_{max}=0.020), CO- (ΔA_{max}=0.012), and NF- (ΔA_{max}=0.008)-treated mice. (+)-PCB 136 bound significantly better to microsomes from PB-treated mice than did (-)-PCB 136 (ΔA_{max}=0.028 and 0.020, respectively). In addition, (+)-PCB 136 bound most effectively, followed by (±)-PCB 136 and (-)-PCB 136, to the other microsomal preparations. The results suggest that (+)-PCB 136 binds more efficiently than (-)-PCB 136 to different hepatic microsomal CYP enzymes, an observation that may explain the enantioselective enrichment of (+)-PCB 136 in mice.

805 IDENTIFYING A UNIQUE HEPATIC GENE EXPRESSION SIGNATURE FOR EXPOSURE TO DIOXIN- LIKE COMPOUNDS.

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The dioxin-like compounds (DLCs), including 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 3,3′,4,4′,5-pentachlorobiphenyl (PCB126), are persistent environmental contaminants which are risks to both public health and the environment. Chronic exposure of female Sprague-Dawley rats to either of these compounds produced hepatocellular adenoma and cholangiocarcinoma, in addition to non-neoplastic hepatic lesions such as diffuse fatty change, multinucleated hepatocytes and bile duct hyperplasia (NTPa 2006; NTPc 2006). In the present study, hepatic gene expression from female rats exposed for 13 or 52 weeks to TCDD (100 ng/kg/day) or PCB126 (1000 ng/kg/day) was assessed using the Affymetrix RGU34A rat genome chip. Toxicogenomic analysis identified genomic changes that may be contributing to the liver pathology observed with chronic exposure to dioxin-like compounds. Analyses of these genomic changes lead to time-independent hepatic gene expression signatures that are unique to TCDD and PCB126. In addition, 41 common genomic biomarkers were identified which are shared between the two AhR ligands. Quantitative real-time PCR was performed on these biomarkers in liver from female rats at 24 hrs after a single dose of TCDD (5 μg/kg, po), in an attempt to assess whether the hepatic gene signature was valid as early as 24 hrs following TCDD exposure. Differentially up and down regulated genes in the hepatic signature were identified as markers of not only subchronic and chronic exposure, but of early responses to dioxin-like compounds. Together, these results can serve to guide future investigations in assessing health risks of dioxin-like compounds and elucidating their mechanisms of action. (Supported in part by National Institute of Environmental Health Science ES09440)

806 DIETHYLSTILBESTROL-LIKE EFFECT OF METABOLITES OF LOWER CHLORINATED BIPHENYLS ON WNT7A GENE EXPRESSION IN A HUMAN ENDOMETRIAL CELL LINE.

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Polychlorinated biphenyls (PCBs) as well as lower chlorinated biphenyls (LCBs) are widespread food and environmental contaminants. Similar to the transplacental carcinogen diethylstilbestrol (DES), neonatal exposure of mice to the PCB mixture Aroclor 1254 disrupted the expression of WNT genes in the developing female reproductive tract. Our previous studies demonstrated that DES disrupted the gene

expression of Wnt7a in human endometrial Ishikawa cells in an estrogen receptor (ER)α- and Wnt5a-dependent way. In contrast to most PCBs, LCBs are susceptible to oxidative metabolism which may generate estrogen-active compounds, potentially more potent as endocrine disrupters than Aroclor 1254. The aim of the present study was to elucidate the potential of selected LCBs to disrupt Wnt7a gene expression in Ishikawa cells. Therefore, the interaction of oxidative metabolites of PCB1, 2, 3, 9, and 12 with ERα in the absence and presence of the female hormone 17β-estradiol was determined in Ishikawa cells using alkaline phosphatase (AlP) as the reporter gene. The influence of the most potent ER-agonist on Wnt5a and Wnt7a mRNA levels was determined by reverse transcription/competitive PCR. The role of ERα was confirmed by RNA interference. PCB1, 2, 3, 9, or 12 did not affect AlP activity while all investigated 4'-hydroxy (HO)-LCBs induced AlP activity. Moreover, the positions of the chlorine atoms affected AlP activity resulting in the following ranking of potency: 4'-HO-PCB9 > PCB1-2',5'-quinone (Q) > 3'-HO-PCB3 = 4'-HO-PCB3 = 4'-HO-PCB12 = PCB12-3',4'-Q (ER agonists); 2',5'-HO-PCB3 = PCB2-2',5'-Q = 2',5'-HO-PCB1 (partial ER agonists); 3',4'-HO-PCB3 (ER antagonist). Like DES, 4'-HO-PCB9 decreased relative Wnt5a and Wnt7a mRNA levels in Ishikawa cells only in the presence of ERα, whereas PCB9 did not affect gene expression of Wnt5a or Wnt7a. Thus, oxidative metabolism may be important for the reprotoxic potential of LCB and PCB mixtures in vivo. (Supported by DFG Le1329/8-1, ES 013661)

807 LOWER CHLORINATED NON-DIOXIN-LIKE PCBs ACT AS PARTIAL AGONIST ON THE $GABA_A$ RECEPTOR AND INHIBIT AROMATASE ACTIVITY.

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Polychlorinated biphenyls (PCBs) have been widely used in a number of industrial and commercial applications such as fluids in transformers, rubber products and plasticizers in paints. Despite their use has been largely prohibited since the 1980's, PCBs are still common in the environment. Non-dioxin-like (NDL) PCBs have been considered less toxic because of their ortho-substituted chlorines, which limit interaction with the aryl hydrocarbon (Ah) receptor. Nevertheless, NDL-PCBs are abundant in food and (human) tissue samples and previous studies have shown that they act as endocrine disruptors by affecting steroidogenesis, including both estrogenic and anti-estrogenic effects. Moreover, NDL-PCBs are potentially neurotoxic by interfering with Ca^{2+} homeostasis and neurotransmitter levels. However, their toxicological properties are still poorly characterized. In the present study, human placental microsomes were used to assess the effect of 20 NDL-PCBs on aromatase activity, a key enzyme in the steroidogenesis pathway. Aromatase activity was measured using the tritiated water-release assay. Of these 20 congeners, only PCB28 inhibited aromatase activity (70% at 25 μM), with an estimated IC_{50} of 1.3 μM. In subsequent experiments, the effects of four common NDL congeners (PCB28, 52, 153 and 180) on human $GABA_A$ receptors expressed in Xenopus oocytes were investigated using the two-electrode voltage clamp technique. None of the tested congeners had antagonistic effects. However, PCB28 and PCB52 (at 1 and 10 μM), were able to potentiate the GABA response under conditions of low receptor occupancy, indicating that NDL-PCBs act as partial agonist of the $GABA_A$ receptor. These *in vitro* findings further add importance to the neurotoxic and endocrine disruptive potential of NDL-PCBs since GABA is not only a major inhibitory neurotransmitter in the brain, but also an important modulator of the endocrine system.

808 INTERACTION OF POTASSIUM PERFLUOROALKYL ACID(PFAA) WITH MODEL AND CELL MEMBRANES: FLUORESCENCE ANISOTROPY STUDY.

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Perfluoroalkyl Acids (PFAA) and their derivatives are persistent environmental pollutants with unknown toxicological potential. We hypothesized that due to their dual lipophobic and hydrophobic structure PFAAs cause adverse effects by interacting with biological lipid membranes. To gain further insights into this possible mechanism of toxicity, we analyzed the effects of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) on phosphatidylcholine bilayers as membrane model, alveolar macrophage, and a human leukemia cell line (HL-60) using fluorescence anisotropy measurement to determine changes in membrane fluidity. Both, PFOS and PFOA, caused a concentration dependent depression of the main phase transition temperature (Tm) and an increased peak width (ΔTw) with dimyristoyl- (DMPC), dipalmitoyl- (DPPC) and distearoylphosphatidylcholine (DSPC). Exposure for 1-3 h to PFOS or PFOA at concentrations up to 372 and 442 μmol/L, respectively, did not induce significant cytotoxicity in either cell line, but there was a significant decreased in fluorescence anisotropy in HL-60 cells above 37 μmol/L of PFOS ($P<0.05$). PFOS also caused changes in membrane fluidity of alveolar macrophages as determined by fluorescence anisotropy measurement. No significant changes in membrane fluidity were observed in HL-60 cells and macrophages exposed to PFOA. We assume that the effects of PFOS are more pronounced compared to PFOA, because of its higher tendency to partition into model and cell membranes. These PFOS- and PFOA-induced changes in both, bilayers of phosphatidylcholine model membranes and membranes of living cells, suggest that interference in the fluidity and thus the functions of cell membranes may be one mechanism by which these fluorinated contaminants cause adverse biological effects. (Supported by the Center for Health Effect of Environmental Contaminants (CHEEC) at the University of Iowa, NIH ES12475 and NSF NIRT 0210517)

809 CYTOCHROME P450 MEDIATED BIOTRANSFORMATION OF 2, 2′, 4, 4′-TETRABROMODIPHENYL ETHER.

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Polybrominated diphenyl ethers (PBDEs) are a class of flame retardants that have become ubiquitous environmental contaminants. 2,2′,4,4′-Tetrabromodiphenyl ether (PBDE 47) is a component of commercial PBDE mixtures and is frequently found in humans and wildlife. The goal of the present study was to investigate the biotransformation of PBDE 47 and to identify the cytochrome P450 (CYP) enzymes involved in metabolite formation. A validated and optimized method to analyze PBDE 47 biotransformation was developed using liquid chromatography-negative electrospray mass spectrometry. PBDE 47 metabolism was assessed initially using liver microsomes prepared from rats treated with phenobarbital (PB), dexamethasone (DEX), 3-methylcholanthrene (MC) and corn oil. Incubation of PBDE 47 with hepatic microsomes from PB-treated rats produced four metabolites: 3-hydroxy-2,2′,4,4′-tetrabromodiphenyl ether (3-OH-BDE47), 4-hydroxy-2,2′,3,4′-tetrabromodiphenyl ether (4-OH-BDE42), 4′-hydroxy-2,2′,4,5′-tetrabromodiphenyl ether (4′-OH-BDE49) and 5-hydroxy-2,2′,4,4′-tetrabromodiphenyl ether (5-OH-BDE47). 4′-OH-BDE49 was the major metabolite with a rate of formation of 24.3 pmol/min/mg protein, followed by 3-OH-BDE47 (10.7 pmol/min/mg protein), 4-OH-BDE42 (5.9 pmol/min/mg protein) and 5-OH-BDE47 (3.1 pmol/min/mg protein). Incubation of PBDE 47 with microsomes from DEX-treated rats produced 3-OH-BDE47 as the major metabolite with a rate of formation of 13 pmol/min/mg protein, followed by 5-OH-BDE47 (5.7 pmol/min/mg protein) and 4′-OH-BDE49 (4.3 pmol/min/mg protein). Incubation of PBDE 47 with hepatic microsomes from MC-treated rats yielded a single metabolite, 4′-OH-BDE49 (17.6 pmol/min/mg protein). No metabolites were detectable when PBDE 47 was incubated with hepatic microsomes from corn oil-treated rats. The specific CYP enzymes involved in hydroxylation of PBDE 47 were investigated using a panel of recombinant rat CYP enzymes. In summary, these results demonstrate that hydroxylation of PBDE 47 in the rat is catalyzed primarily by CYP2B and CYP3A enzymes.

810 PARAOXONASE (PON1) ACTIVITY IN RATS AFTER EXPOSURE TO POLYCHLORINATED BIPHENYLS (PCBs).

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Paraoxonase (PON1, EC 3.1.8.1) is synthesized in the liver and secreted into the blood. PON1 is an antioxidant, which protects HDL and LDL against lipid peroxidation, a major risk factor in atherosclerosis, cardiovascular and neurodegradative disease, and cancer. The objective of this study was to determine if PCBs influence plasma PON1 activity. Rats were injected ip with 100 μmol/kg of PCB3, 4-OH-PCB3, PCB47, PCB126 (males only), PCB136, or a cytochrome P450 inducer, or corn oil (control). The plasma PON1 activities were measured spectrophotometrically using two substrates, paraoxon and phenylacetate. In male rats plasma PON1 activities were significantly reduced, up to 54%, by 4-OH-PCB3, PCB47 and PCB136 compared to the control measured with either substrate, and by PCB126 (-23%) measured with phenylacetate. Female control rats had a 15 or 34 % (phenylacetate and paraoxonase, respectively) higher PON1 activity compared to males and the plasma PON1 activity was only reduced significantly (49-53%) by PCB136 (phenylacetate) and PCB3 (both substrates). Both CYP1A inducers (β-NF and 3-MC) reduced plasma PON1 activity in male rats, but only 3-MC in female rats. Phenobarbitol (CYP2B inducer) and dexamethasone (CYP3A inducer) had no significant effect on PON1 activity in either gender. Except PCB126 none of the PCB congeners tested is known to be a strong CYP1A inducer, which excludes this activity as possible mechanism for the observed effects. Our findings indicate that PCBs can lower the plasma PON1 activity and may thereby contribute to the risk of cardiovascular and other diseases. (Supported by NIEHS P42ES013661 and DOD DAMD17-02-1-0241).

811 INTERACTIONS OF NON-DIOXIN-LIKE 2, 2', 4, 4', 5, 5'-HEXACHLOROBIPHENYL (PCB 153) WITH MEMBRANE-ASSOCIATED AND INTRACELLULAR SIGNALING PATHWAYS IN LIVER EPITHELIAL CELLS.

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Noncoplanar PCB 153 is a prevalent environmental and food-web contaminant, which may act as a tumor promoter or a disruptor of several endocrine pathways. However, the exact mechanisms underlying its toxicity are far from a being completely understood. In this study, performed in liver epithelial WB-F344 cells, we found that PCB 153 suppressed levels of several key junction and adherens proteins. Mitogen-activated protein kinases ERK1/2 were transiently activated by PCB 153, which could be associated with increased degradation of gap-junctional protein connexin 43, but not with changes in proliferative rate. Moreover, fluidity of plasmatic membrane and perturbations in lipid signaling were detected, such as a massive release of arachidonic acid. However, neither arachidonic acid nor C2-ceramide were able to induce apoptosis. PCB 153 moderately interfered with PAH- or UV-induced apoptosis. Although PCB 153 triggered multiple intracellular events, their causal relationships remain to be established, especially those leading to disruption of cell-to-cell communication and modulation of apoptosis induced by genotoxic insult. Changes in gene expression induced by PCB 153 might help to explain contributions of individual signaling pathways and transactivated transcription factors to the processes associated with tumor promotion. [Supported by the Czech Ministry of Agriculture, grant No. 0002716201.]

812 DECACHLOROBIPHENYL (PCB209) LACKS ENZYME INDUCTION POTENTIAL, GENETIC TOXICITY, AND ENDOCRINE ACTIVITY.

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PCB209 is a fully chlorinated, non-coplanar biphenyl found as a by-product of some waste streams. To demonstrate that PCB209 does not exhibit the effects of coplanar PCBs it was tested for P450 enzyme induction, genetic toxicity, and endocrine activity. PCB209 did not demonstrate significant induction of P450 CYP1A, 2A, 2B, 3A, or 4A in Arochlor-induced rat hepatocytes. PCB209 showed no evidence of mutagenicity in *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 or *E. coli* strain WP2uvrA, in the presence or absence of metabolic activation. PCB209 was negative for forward mutations at the thymidine kinase locus of L5178Y mouse lymphoma cells in the presence and absence of metabolic activation. PCB209 did not induce biologically relevant increases in micronucleated polychromatic erythrocytes in mouse bone marrow *in vivo*. PCB209 did not induce estrogenic effects when administered by gavage to ovariectomized adult female rats at 500 and 1000 mg/kg for 4 days. There was no mortality, clinical signs of toxicity, test-substance related gross observations, substance-related effects on vaginal cytology, or effects to liver or uterus weight during the study. PCB209 did not produce alterations consistent with endocrine-modulating activity in intact adult male rats when administered by gavage at 500 and 1000 mg/kg for 15 consecutive days. There was no mortality, clinical signs of toxicity, test substance-related effects on testes, epdidymides, or thyroid gland weights or microscopic observations, and no effects to serum concentrations of testosterone, DHT, prolactin, LH, FSH, or TSH. Liver weights were increased and serum thyroid hormone levels were decreased, but represented a transient non-biologically significant response in the absence of other changes in the liver and thyroid. PCB209 did not induce P450 enzymes, was not genetically active, and did not show endocrine activity in male and female rats at high administered doses.

813 3, 3', 4, 4'-TETRACHLOROBIPHENYL (PCB77) ALTERS DIETARY FATTY ACID ACTIVATION OF PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR-ALPHA (PPARα) AND INDUCTION OF ITS RESPONSE GENES IN MOUSE LIVER.

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Exposure to persistent organic pollutants, such as polychlorinated biphenyls (PCBs) and dioxins, has been shown to induce a wide variety of toxic effects. Epidemiological studies have shown positive correlations between exposure to persistent organic pollutants and increased serum levels of hepatic enzymes. In human populations, prolonged intake of diets high in fat/calories can promote inflammatory diseases, including obesity, and fatty liver diseases (including steatosis and cirrhosis). The hepatic responses to dietary fatty acid exposure are orchestrated, in part, by the PPARα pathway. To determine how high fat diets can affect PCB-induced pathological liver responses, male mice (C57/BL6) were exposed to diets enriched with safflower oil (20 or 40% fat calories) for a total of four months. During the last two months of the study, mice were injected (i.p.) every other week with PCB77 (170 μmol/kg) or vehicle (safflower oil). Liver tissues were sampled, and RNA was extracted to measure mRNA abundance through expression microarray. The results are being validated by real time-PCR. Gene expression patterns demonstrate that the high fat diet (40%) induced expression of genes that play a critical role in fatty acid uptake and metabolism, and that are regulated by PPARα signaling. Treatment with PCB77 blocked induction of these genes and reduced expression of PPARα. These results suggest that by inhibiting PPARα expression and function, PCB77 exposure can accelerate the detrimental effects of a high fat diet associated with inflammatory diseases. Supported by grants from NIEHS, NIH (P42ES07380) and the University of Kentucky AES).

814 ANALYTICAL METHOD DEVELOPMENT FOR THE SIMULTANEOUS EXTRACTION AND ANALYSIS OF BISPHENOL A (BPA), BIS-GMA, BADGE, BPAHPE, BPADM AND TEGDMA IN HUMAN URINE AND SALIVA.

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Bisphenol A (BPA) is widely used in the production of resins and plastics that are in turn used make items such as baby bottles and as a lining for canned foods. Some derivatives of BPA have also been used as a dental composite which could decompose into BPA and increase exposure. Recent studies have raised concerns regarding the toxicological effect that low-doses of BPA might have on humans. These concerns center around the high daily exposure an individual has to BPA and its derivative products and the possibility of persistent levels of BPA in humans. The Midwest Research Institute is currently providing analytical support to the Environmental Toxicology Program on a study of BPA and five compounds with structural similarities. The other five compounds include four derivative or possible metabolites of BPA (Bis-GMA, BPADM, BPAHPE and BADGE) and one compound used as a cross-linking reagent in polymerization reactions (TEGDMA). The present work includes the analytical method developed to simultaneously extract and analyze for BPA and five related compounds from human urine and saliva using C18 solid Phase Extraction and liquid chromatography/mass spectrometry. The average recovery of the six analytes using the developed method ranged from 19.6 to 106.4 percent. Analysis of the data suggested that the low percent recoveries for some compounds were due to compound instability. The percent recoveries for most of the six compounds were improved when stored in a refrigerator or freezer with the exception of BPADM which proved unstable under any storage conditions in either matrix. The optimal analytical concentration range and the lower limit of detection were also explored for each compound. With the noted exception of BPADM, the developed method proved to be suitable for the simultaneous extraction and LC/MS analysis of the analytes.

815 PBDE CONGENERS ARE CAR ACTIVATORS.

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Polybrominated diphenyl ethers (PBDEs) belong to a family of brominated flame retardants. PBDEs have emerged as a major environmental pollutant due to their persistent and bioaccumlative nature. Human exposure occurs chronically through the ingestion of contaminated food products as well as inhalation of PBDE-containing dust particles. PBDE47, 99 and 209 are the most common congeners found in human tissue samples. PBDE congeners are toxic in rodents and have been shown to induce cytochrome P450s (CYPs) and disrupt thyroid hormone homeostasis. We have shown previously that PBDEs are PXR activators. However, the mechanism by which PBDEs induce the cyp2b family is not clear. In the current study, we have showed that PBDE congeners induced cyp2b10 in mice. In addition, luciferase reporter gene assays demonstrated that PBDEs activate both mouse and human constitutive androstane receptor (CAR), a member of the nuclear receptor superfamily. In conclusion, PBDEs are activators for xenobiotic nuclear receptors, which can be a mechanism responsible for the induction of P450 enzymes and disruption of thyroid hormone metabolism in vivo. In conclusion, PBDEs are activators for xenobiotic nuclear receptors, which can be a mechanism responsible for the induction of P450 enzymes and disruption of thyroid hormone metabolism in vivo.

816 CONGENER CLASS AND CONGENER-SPECIFIC RELATIVE POTENCY VALUES FOR DIOXIN AND DIOXIN-LIKE CHEMICALS DETERMINED IN NORMAL HUMAN EPIDERMAL KERATINOCYTES.

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A report by Zeiger et al in 2001 showed that the relative potency (REP) value of PCB 126 determined in the human hepatocyte cell line, HepG2, was much lower than the REP values determined in either the rat hepatocyte cell line, H4IIE, or in primary cultures of rat hepatocytes. In 2005, Silkworth et al confirmed and expanded on this observation, reporting that the cross species EROD-based REP value for PCB 126 in human hepatocytes was 100-fold less than the REP value in rat hepatocytes, supporting the concept that humans are less sensitive than rats. If accepted by risk assessment for human health risk, that work would support a toxic equivalency factor (TEF) for PCB 126 of 0.001, a value 100 times lower than the current value of 0.1. Historic difficulties in measuring enzyme induction in primary cultures of hepatocytes limit the interpretation of these studies. To confirm the REP value of PCB 126 in other human cells and to determine whether this result was reflective of all dioxin-like compounds with lower REP values or rather a congener class or congener-specific result, we used primary cultures of normal human epidermal keratinocytes (NHEKs), known targets for dioxin toxicity in humans. The EC50-based REP values for CYP1A1 mRNA induction were determined for PCB 126, TCDD, and 2,3,7,8-tetrachlorodibenzo-p-furan (TCDF). Our results demonstrate that in NHEKs, PCB 126 has a REP value of 0.002, compared to 1 of TCDD, the same as the within-species value reported earlier for human hepatocytes. This value is 50 times less than the TEF value of 0.1, assigned to it by WHO2005. TCDF had a REP value of 0.16, which is 1.6 times its assigned value. These results from primary cultures of NHEKs support the findings of others, namely that humans are less sensitive than rats to PCB 126. Since the current TEF is rodent based, but used for humans, modern risk assessment should incorporate a cross species factor.

817 DIVERGENT DISRUPTION OF SEXUALLY DIMORPHIC BASAL EXPRESSION OF STEROID METABOLISM GENES IN PCB AND DIOXIN EXPOSED SPRAGUE-DAWLEY RATS.

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Chronic exposure of Sprague-Dawley (SD) rats to either 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or Aroclor 1254 results in female-selective induction of hepatic tumors. A likely mode of action (MOA) for this response is promotion of spontaneously-initiated hepatocytes. Previous studies in our laboratory have elucidated key events for this MOA as: (1) disregulation of cytochrome P450 activity; (2) increased estrogen metabolism leading to reactive oxygen species (ROS) production; and, (3) ROS-mediated cell proliferation. The current study determined acute gender-specific gene expression changes in Aroclor 1254- and TCDD-dosed SD rats related to these key events. Aroclor 1254 at 0.6, 6.0 and 60 mg/kg body weight and toxic equivalent (TEQ) doses of TCDD (0.3 and 3.0 ug/kg) were orally administered to rats for 3 consecutive days and on day 4 gene expression was determined using microarrays. Array analysis revealed that most gender differences in response differed merely in the magnitude of change and did not involve sex-specific alterations. However, a preponderance of female-dominant genes were repressed and male-dominant genes induced following exposure to both TCDD and Aroclor. Since many of these sexually-dimorphic genes are involved in steroid metabolism, exposure to either TCDD or Aroclor 1254 would certainly disrupt hepatic estrogen metabolism. Furthermore, disruption (direct or indirect) of hepatic growth hormone (GH) signaling cannot be ruled out as an event in the proposed MOA since GH controls the basal expression of most of these genes and both chemicals repressed the GH receptor gene. Thus, gene expression changes observed in the current study were in agreement with the proposed MOA for these compounds in SD rats. The relevance of this MOA to humans will require further investigation.

818 MUTAGENICITY OF 3-METHYLCHOLANTHRENE, 4-MONOCHLOROBIPHENYL (PCB3), AND ITS METABOLITE 4-OH-PCB3 IN THE LUNG OF MALE TRANSGENIC BIGBLUE® RATS.

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Recent findings of high levels of PCBs in indoor and outdoor air open the question of the possible health consequences of exposure to these predominantly lower chlorinated biphenyls. Unlike the higher chlorinated biphenyls, lower chlorinated congeners are more readily metabolized to reactive and potentially harmful intermediates, which might lead to interaction with the DNA, mutagenesis, and possibly cancer initiation. The goal of this study was to assess the mutagenicity of PCB3 in the lung of rats. Male BigBlue® 334 Fisher transgenic rats, which carry the bacterial *lacI* gene as target of mutagenicity, were given intraperitoneal (IP) injections of corn oil as a negative control, 3-methylcholanthrene (3-MC) as a positive control, 4-monochlorobiphenyl (PCB3) or its metabolite 4-hydroxy-PCB3 (4-OH-PCB3) weekly for 4 weeks. Lungs were removed and analysis performed to determine mutant frequency, mutation spectrum, and pathological changes. 3-MC caused a 15-fold increase in mutant frequency and an increase in transversion type mutations. There was a 2-fold increase in the mutant frequency after treatment with PCB3 and 4-OH-PCB3 that was not statistically significant. However, a clear shift in the mutation spectra, especially with PCB3, and an increase in mutations in areas of the gene outside of the hotspot region for spontaneous mutations (bp 1-400) were observed. These results suggest that PCB3 and possibly 4-OH-PCB3 are mutagenic in the lung of rats and demonstrate for the first time the early occurrence of that type of mutation in 3-MC-lung tissue that was identified in Ki-ras oncogenes of lung tumors from 3-MC-exposed mice. (Supported by P42ES013661 and DAMD17-02-1-0241)

819 AGE DEPENDENT CARDIOTOXICITY OF 2, 3, 7, 8 TETRACHLORODIBENZO-*P*-DIOXIN IN ZEBRAFISH.

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Zebrafish embryos exposed to 2,3,7,8 tetrachlorodibenzo-*p*-dioxin (TCDD) develop profound cardiac and vascular defects, including pericardial and yolk-sac edema, heart looping defects and reduced peripheral blood flow. To test whether the effects of TCDD are age dependent, we exposed zebrafish to 0.2 or 0.7 ng/mL TCDD at different stages of development from 1 hour post fertilization to 37 days post fertilization. TCDD exposed zebrafish displayed reduced mortality with age, demonstrating reduced toxicity. ^{3}H-TCDD uptake revealed that dioxin absorption increases with age, signifying that resistance was not the result of reduced exposure. Previous work from our laboratory analyzed TCDD induced transcript changes in the embryonic zebrafish heart, and identified reductions in cell cycle transcripts and alterations in genes involved in cardiac development and contractility. To test if the cardiac transcriptional response to TCDD is age dependent, we exposed juvenile zebrafish to vehicle or a lethal concentration of TCDD (1 ng/mL) for one hour and collected heart samples at 2 and 12 hours post exposure and at 2 weeks post exposure for microarray analysis. Our results show that a core battery of cardiac transcripts, including mediators of xenobiotic metabolism, are induced by TCDD independent of age. However, large differences in transcriptional responses between the embryonic and juvenile hearts are also seen. In contrast to the embryonic heart, juvenile hearts displayed reductions in cell adhesion and tight junction transcripts, and up-regulation of transcripts associated with hypoxia and insulin resistance. No changes in cell cycle transcripts were observed in the juvenile hearts. We conclude that the effects of TCDD exposure are age dependent and likely due to age associated changes in transcriptional response.
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820 CHLORDECONE PRETREATMENT PROMOTED UP-REGULATION OF SCAVENGER RECEPTOR B2 IN MOUSE HEPATIC MICROSOMES.

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Previous work has shown that the pretreatment of male C57BL/6 mice with low doses of the organochloride insecticide chlordecone (CD) stimulated biliary excretion of exogenous cholesterol (CH) up to 2-fold. Increased biliary excretion was not associated with changes in ATP-binding cassette transporter G8 (ABCG8) or scavenger receptor class B type 1 (SR-B1). Scavenger receptor class B type 2 (SR-B2) was identified as a splice variant from the SR-B1 gene and is expressed in a variety of tissues. Although the function of SR-B2 is not clear it was proposed to play a role in CH homeostasis and trafficking that was distinctly different than SR-B1 (Eckhardt, E. R. M., et al., J. Biol. Chem.279:14 14372-14381, 2004) . The present study was undertaken to determine if changes in SR-B2 protein were associated with CD-induced stimulation of CH biliary excretion. Three days after intraperitoneal injections with corn oil vehicle or CD (5 or 15 mg/kg in corn oil) mice were killed and liver homogenates were subjected to differential centrifugation in order to obtain microsomal, cytosolic and crude membrane fractions. Westerns blots

were performed on the microsomal and crude membrane fractions using commercial antibodies to SR-B2. Cytosol was subjected to a relative, quantitative proteomic analysis (iTRAQ). Analysis of the western blots showed at least a 30% increase in SR-B2 with CD treatment. CD is a mixed agonist for estrogen receptor alpha (ERα), steroid and xenobiotic receptor (SXR) and an effective ERβ antagonist and 17β-estradiol has been shown to up-regulate SR-B2 in Hep G2 cells and in rat liver (Graf, G. A, et al., J. Lipid Res. 42:1444-1449, 2001). This study indicates a possible mechanism by which organochlorine endocrine disruptors might act to alter cholesterol homeostasis in the mouse.

821 NOVEL HYDROPHOBICITY RULER APPROACH FOR DETERMINING THE OCTANOL/WATER PARTITION COEFFICIENTS OF VERY HYDROPHOBIC COMPOUNDS *VIA* THEIR POLYMER/SOLVENT SOLUTION DISTRIBUTION COEFFICIENTS.

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We have developed a novel hydrophobicity ruler approach for determining the octanol/water partition coefficients of very hydrophobic compounds. The hydrophobicity ruler approach is an indirect method that measures the polymer/solvent solution distribution coefficients (logKp/s) of the reference and unknown compounds. The logKp/s values of the unknown compounds can be calibrated to their logKo/w values via the correlation of the logKp/s values of the reference compounds with their logKo/w values. A water miscible organic solvent was used to increase the solubility of the very hydrophobic compounds in the aqueous solution. The solvent reduces the hydrophobicity scale of the very hydrophobic compounds and reduces the absorption amounts of the very hydrophobic compounds into the PDMS disk, so that compounds with very wide logKo/w range can be measured in one measurement and the coexisting compounds will not interfere each other due to their over saturation. A one-compartment model was proposed to describe the absorption kinetics. Two constants introduced into the mathematical model can be obtained by regression of the absorption data sampled over a periods of time before equilibrium. Polydimethylsiloxane (PDMS)/methanol aqueous solution and a series of 21 PCB carcinogens were used to demonstrate the approach. The PCB compounds with known experimental logKo/w values served as reference compounds, while the PCB carcinogens without known logKo/w values were determined. The distribution coefficients (logKp/s), uptake and elimination rate constants were obtained from the two constants for each compound (reference or unknown). The correlation of the logKp/s values of the reference PCB compounds with their logKo/w values was linear (logKo/w = 3.232 logKp/s -0.376, R2=0.95). The critical experimental factors, the advantages and limitations of the hydrophobicity ruler approach were discussed.

822 HEPATIC TRANSPORTER AND DRUG METABOLISM ENZYME EXPRESSION IN HUMANS WITH NON-ALCOHOLIC FATTY LIVER DISEASE.

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Severe adverse drug reactions among patients in the United States number as high as two million per year. Less than 20% of these cases are due to genetic polymorphisms, whereas, the vast majority of adverse drug reactions are likely due to environmental factors such as age, nutrition, or disease state. Non-alcoholic fatty liver disease (NAFLD) encompasses a spectrum of diagnoses ranging from simple fatty liver (SFL) to non-alcoholic steatohepatitis (NASH) and eventual progression to cirrhosis. Because changes in hepatic transporter and cytochrome P450 (P450) expression can lead to variability in drug disposition, either in the form of drug toxicity or decreased therapeutic effectiveness, the purpose of this study was to determine whether expression of these important genes is altered in human livers diagnosed with mild to severe NAFLD. Human liver samples diagnosed as normal, SFL, NASH with fatty liver (early stage) or NASH without fatty liver (end stage NASH-cirrhosis) were provided by the NIH funded Liver Tissue Procurement and Distribution System. While no significant changes were observed in SFL livers, the expression of several hepatic transporters and P450s was significantly altered in human NASH livers. CYP2E1 mRNA levels were decreased in human livers diagnosed with early stage and end stage NASH, respectively, while CYP4A mRNA levels were increased only in early stage NASH. Human livers diagnosed with early stage NASH showed significant increases in BCRP, MRP1, MRP4 and MRP5 hepatic transporter mRNA levels relative to normal human liver samples, while end stage NASH livers displayed increases in MRP1, MRP4 and MRP5. In addition, protein expression of MRP2, 3 and 4 was increased in both stages of NASH. These data suggest that the expression of hepatic transporters and P450 enzymes is altered during the more severe stages of NAFLD, and may result in impairment of hepatic metabolism and disposition of clinically relevant drugs administered to these patients. (DK068039, ES07091, AT002842)

823 ISOFORMS OF ALANINE AMINOTRANSFERASES IN HUMAN TISSUES AND SERUM.

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Serum alanine aminotransferase (ALT) is used as a clinical marker of hepatotoxicity. Two forms of ALT have been identified in the genome of most species studied, ALT1 and ALT2. A shorter form of ALT2 also exists due to alternative splicing (ALT2_2). The cellular and tissue distributions of the different ALT proteins have not been characterized in humans, and their relative contributions to serum enzyme activity is unknown. Here, we describe the development of novel, isoenzyme specific ALT1 and ALT2 antibodies and the expression of the enzymes in human cells and organs. In normal human tissue, high expression of ALT1 was found in liver, skeletal muscle and kidney and low levels in heart muscle and not detectable in pancreas. High ALT2 reactivity was detected in heart and skeletal muscle, while little or no ALT2 expression was found in liver or kidney. Using immunohistochemistry, strong ALT1 reactivity was found in hepatocytes, renal tubular epithelial cells and in salivary gland epithelial cells, while ALT2 was expressed in adrenal gland cortex, neuronal cell bodies, cardiac myocytes, skeletal muscle fibers and endocrine pancreas. We also show that protein and mRNA expression of ALT1 in cultured primary hepatocytes can be induced by different pharmaceuticals, PPAR agonists, and the mechanism behind this effect is increased transcription of the ALT1 gene. In addition, immunoprecipitation using ALT antibodies on normal human serums showed ALT1 to be mainly responsible for basal ALT activity. Together, these results points to a differential expression of ALT1 and ALT2 in human organs, and the intracellular levels of ALT1 may be regulated by compounds or other metabolic factors. This substantiates a need for further investigations regarding the possible impacts on ALT measurements in clinical practice.

824 ADAPTIVE REGULATION AFTER HEPATOCYTE-SPECIFIC LOSS OF NADPH-CYTOCHROME P450 REDUCTASE (CPR) IN MICE.

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NADPH-cytochrome p450 reductase (Cpr) is an essential component of cytochrome p450s (Cyps). Mice with hepatocyte-specific Cpr knockout develop normally and are able to breed and survive with complete loss of hepatic Cyp activity. The purpose of the present study was to determine the underlying mechanisms by which hepatocyte-Cpr-null mice survive, by evaluating regulation of mRNA expression of other phase-I and -II enzymes, as well as transporters in liver, kidney, and small intestine of adult hepatocyte-Cpr-null mice. In livers of hepatocyte-Cpr-null mice, there is a marked increase in the mRNA expression of phase-I enzymes (aldehyde dehydrogenase 1a1, 1a7, 3a2; carboxylesterase 1b2, 2a6, and 2a12; as well as antioxidant enzymes heme oxygenase-1, NAD(P)H:quinone oxidoreductase-1, and epoxide hydrolase), and phase-II enzymes (UDP-glucuronosyltransferase 1a9; glutathione S-transferase a1/2, m3, m4, m6, t1, and t3; and sulfotransferase 1a1 and 1d1), as well as drug transporters (organic anion-transporting polypeptide [Oatp] 1a4, organic cation transporter 3, multidrug and toxin extrusion-1, multidrug resistance transporter [Mdr] 1a, multidrug resistance-associated protein [Mrp] 3 and 4). In contrast, in kidneys of hepatocyte-Cpr-null mice, mRNA levels of Cyp3a11 and 4a14 are decreased, and mRNA expression of transporters is not altered except for Oatp1a1 and Mrp3, which are down-regulated. In small intestine of hepatocyte-Cpr-null mice, mRNA expression of Cyp3a11 and transporters including peptide transporter 1, organic solute transporter α/β, Niemann-Pick C1-Like 1 (Npc1L1) and Mdr1a are up-regulated. In conclusion, in hepatocyte-Cpr-null mice, adaptive regulation of other phase-I and -II drug-metabolizing enzymes and transporters in liver and intestine helps to maintain whole body homeostasis and survival. (Supported by NIH grants ES-09649, ES-09716, ES-013714, and RR021940.)

825 CHARACTERIZATION OF MICE NULL FOR LIVER-SPECIFIC UPTAKE TRANSPORTER ORGANIC ANION TRANSPORTING POLYPEPTIDE 1B2 (OATP1B2).

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The liver-specific importer Oatp1b2 transports a large variety of chemicals. Oatp1b2-null mice were engineered by homologous recombination and their phenotype was characterized. Hepatic mRNA and protein expression of transporters were determined with branched DNA signal amplification assay and Western blot,

respectively. Oatp1b2 protein was absent in liver of Oatp1b2-null mice. Compared to wild-type, Oatp1b2-null mice had higher hepatic mRNA expression of Oatp1a4 but lower organic anion transporter 2. Oatp1b2-null mice develop normally and breed well. However, adult Oatp1b2-null mice had moderate conjugated hyperbilirubinemia. After intra-arterial injection of unconjugated bilirubin, Oatp1b2-null mice had as much as 5-fold higher blood levels of conjugated bilirubin than wild-type mice. Oatp1b2-null mice were completely resistant to the hepatotoxicity induced by the cyanotoxin microcystin-LR and mushroom toxicant phalloidin (an Oatp1b2-specific substrate identified in vitro). The critical role of Oatp1b2 in mediating hepatotoxicity of phalloidin was further confirmed by the markedly attenuated cholestasis in Oatp1b2-null mice after intra-arterial injection of phalloidin. After intra-arterial injection of dibromosulfophthalein (DBSP), a non-metabolizable organic anion used as a liver function assay, blood concentrations of DBSP were much higher, and cumulative biliary excretion of DBSP was lower in Oatp1b2-null mice compared to wild-type mice. Additionally, Oatp1b2-null mice were partially resistant to cholestasis induced by estradiol-17β-D-glucuronide. In conclusion, Oatp1b2 plays a key role in determining hepatic uptake of conjugated bilirubin, phalloidin, microcystin, DBSP, and estradiol-17β-D-glucuronide. Oatp1b2-null mice are useful in elucidating the physiological role of Oatp1b2 and its human orthologs OATP1B1 and OATP1B3. (Supported by NIH grants 5R01ES009649, R01ES09716, R01ES013714 and RR021940.)

826 ROLE OF HEPATIC LIPASE IN ACROLEIN-INDUCED DYSLIPIDEMIA IN MICE.

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Acrolein is a highly reactive aldehyde pollutant detected in high levels in automobile exhaust, tobacco smoke, and as a natural constituent of several foods. We have found that a single oral administration of acrolein causes a dose-dependent (0.1-5 mg/kg) increase in plasma cholesterol (~50%) and triglycerides (>100%) in adult male C57Bl/6 and apoE-null mice within 24h of treatment. Moreover, chronic acrolein feeding (2.5 mg/kg/d; 8 wks) induced sustained hypercholesterolemia, but not hypertriglyceridemia, in apoE-null mice indicating that acrolein chronically affects cholesterol synthesis and/or clearance. Based on these observations, current investigations were designed to test whether acute acrolein affects cholesterol synthesis or clearance by altering the expression or the activity of hepatic genes. Changes in hepatic genes were measured by qRT-PCR of: 1) 'master switches' for fatty acid oxidation (SREBF-1) and cholesterol synthesis (SREBF-2); 2) genes encoding proteins involved in lipoprotein synthesis and clearance (apoB, HMG-CoA reductase, hepatic lipase [HL]). Adult male C57 mice were fed acrolein (5 mg/kg) and livers were harvested at 6 and 12h but before changes in plasma lipids. Feeding acrolein led to a significant decrease in HL transcript levels (-47±14%; n=4,4) and plasma HL activity (-43±13%, n=6,5) 12h after acrolein treatment when compared with vehicle-fed controls. Acrolein also decreased SREBF-1 transcript levels (-85±4%) at 6, but not at 12h after treatment, while other genes were unaffected. The hepatic cholesterol content (2.80±0.05) of acrolein-fed mice was higher than that of controls (2.69±0.05 μg/mg wet weight; n=5,6) while liver triglyceride content was unaltered by acrolein. These data suggest that dyslipidemia induced by acrolein-feeding could be attributable in part to decreased lipoprotein clearance induced by the down regulation of hepatic lipase. Sustained dietary or environmental exposure to acrolein may be associated with clinical states of dyslipidemia and thereby contribute to the risk of cardiovascular diseases. This work supported by PO1 ES11860.

827 INDUCTION OF BILE ACID TRANSPORTERS AND NUCLEAR RECEPTORS BY BILE ACIDS.

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Bile acids (BAs) act as hormones to regulate the homeostasis of lipids and bile acids by activation of nuclear receptors farnesoid X receptor (FXR) and pregnane X receptor (PXR). The purpose of this study was to determine whether BAs regulate their own transporters as well as nuclear receptors. Mice were fed individual BAs, including cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), lithocholic acid (LCA), and ursodeoxycholic acid (UDCA), at concentrations of 0.1, 0.5, 1.0, or 1.5% (W/W), or cholestyramine (CSA) at 2% in the diets for one week. At the lowest concentration (0.1%), all BAs except for LCA, were not hepatotoxic, as determined by serum ALT. UDCA and CSA were not toxic at any dose tested. Branched DNA assays showed that hepatic Ntcp and Bsep mRNA expression in mouse livers was induced by BAs at the lowest dose, but not altered in mice fed UDCA or CSA. Similar induction of Oatp1a1, 1a4, and 1b2 mRNA were observed after bile acid feeding. Western blot assays indicated that Ntcp and Bsep proteins were induced after bile acid feeding. Immunohistofluorescence indicated that the location of Ntcp was in the basolateral and Bsep in the canalicular region. All bile acid feedings reduced Cyp7a1 mRNA to similar levels, suggesting decreased synthesis of BAs. LC-MS/MS analysis demonstrated that the concentrations of total and taurine conjugated BAs increased in livers of mice fed bile acids. As expected, small heterodimer partner (SHP) was induced by bile acid feeding. Surprisingly, FXR, PXR and constitutive androstane receptor (CAR) were also induced by BAs. In conclusion, at non-toxic doses (0.1%), BAs induce hepatic gene expression of both bile acid transporters and nuclear receptors. (Supported by NIH grants: R01ES09649, R01ES09716, R01ES013714, and RR021940.)

828 GENDER- AND AGE-DEPENDENT HEPATIC GENE EXPRESSION DUE TO RXRα DEFICIENCY.

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The incidence of cancer including liver cancer is different between genders. The incidence of these diseases also increases due to aging. Retinoids are commonly used to treat and prevent cancers. The action of retinoids can be mediated through its receptors including retinoid X receptor α, which is highly expressed in the liver. Aberrant RXRα signaling has been implicated as a possible mechanism for the development of hepatocellular carcinoma. To study the role of hepatic RXRα in regulating liver gene expression in a gender- and age-dependent manner, the hepatic gene expression pattern was compared between hepatocyte RXRα-deficient and wild type mice. Male and female mice of 6 (mature) - and 24 (aged)-month old were used. Genespring 7.1 and IPA were used for data analysis. The threshold for a significant change was set greater than 2-fold. Genes were classified into five categories based on their roles in carcinogenesis and disease process, which are apoptosis genes, stress inducible genes, cell growth regulatory genes, cell-cell or cell-matrix interaction genes, and immune response genes. In 6-month old mature mice, hepatocyte RXRα deficiency resulted more changes (both number and fold) in gene expression in male than female mice. For example, in male mice, many proto-oncogenes and tumor suppressor genes were down-regulated due to RXRα deficiency that include Fos, Jun, Myc ; BTG2, EGR1, and KLF6. In contrast, except for KLF6, none of above genes changed their expression level in female RXRα deficient mice. In aged mice, the expression pattern was reversed and females showed more changes than males. In both genders, hepatocyte RXRα deficiency caused more deviations in the expression of immune response genes. This gender- and age-dependent gene expression pattern due to disrupted RXRα signaling might in part explain gender- and age-dependent incidence of many diseases. (NIH R01 CA053596).

829 ANIT-TREATED FXR-NULL MOUSE LIVERS EXHIBIT MORE SINGLE CELL DEGENERATION DUE TO IMPAIRED INDUCTION OF EFFLUX TRANSPORTERS.

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Alpha-naphthyl isothiocyanate (ANIT) is a hepatotoxicant which produces acute intrahepatic cholestasis. Farnesoid X Receptor (FXR) and Pregnane X Receptor (PXR) are two major bile acid sensors in liver. The purpose of this study was to characterize the regulation of hepatic transporters by FXR and PXR in ANIT-induced liver injury. The mRNA expression of three hepatic transport systems was examined by bDNA assay in wild-type, FXR-null, and PXR-null mice: 1) canalicular efflux transporters: Bsep, Mrp2, and Mdr2; 2) basolateral uptake transporters: Ntcp, Oatp1a1, Oatp1a4, and Oatp1b2; and 3) basolateral efflux transporters: Ostα, Ostβ, Mrp3, and Mrp4. Transporter proteins were analyzed by immunofluorescence. The present findings indicate that livers from wild-type and PXR-null mice have a comparable degree of multi-focal necrosis 48h after ANIT treatment. In contrast, ANIT-treated FXR-null livers have less necrotic foci, but express marked single-cell degeneration and necrosis of the remaining parenchyma (H & E). Serum biomarkers alanine transaminase (ALT) and alkaline phosphatase (ALP) increase in all genotypes after ANIT treatment, with higher ALP in FXR-null mice. ANIT induces all three canalicular transporters (Bsep, Mrp2, and Mdr2) in wild-type mice, induces Bsep and Mdr2 in PXR-null mice, but fails to induce any canalicular transporter in FXR-null mice. ANIT decreases basolateral uptake transporters (Ntcp, Oatp1a1, and Oatp1b2) in livers of all three genotypes of mice. ANIT induces the basolateral efflux transporters Ostβ and Mrp3 in livers of wild-type and PXR-null mice, but fails to induce Ostβ in FXR-null mice. In conclusion, FXR deficiency causes a unique injury pattern of ANIT-induced intrahepatic cholestasis, which is likely a result of impaired induction of efflux transporters in FXR-null liver upon ANIT insult. (Supported by NIH grants ES-09649, ES-09716, ES-07079, and ES013714.)

830 UPREGULATION OF EARLY GROWTH RESPONSE FACTOR-1 BY BILE ACIDS REQUIRES EGF RECEPTOR ACTIVATION.

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Cholestasis is a condition that results when excretion of bile acids from the liver is interrupted. Liver injury occurs in both humans and animals with cholestasis. Recent studies have shown that inflammation is required for injury, and our studies have shown that early growth response factor-1 (Egr-1) is required for development of inflammation in liver during cholestasis. Furthermore, Egr-1 is upregulated in primary mouse hepatocytes exposed to pathological concentrations of bile acids. What remains unclear is the mechanism by which bile acids up-regulate Egr-1. Two mechanisms by which bile acids regulate gene expression are through the Farnesoid X Receptor (FXR) or by activation of the epidermal growth factor receptor (EGFR) signaling through the mitogen-activated protein (MAP) kinase cascade. To elucidate the mechanism by which bile acids up-regulate Egr-1, primary mouse hepatocytes were isolated from wild type and FXR knockout mice and treated with either deoxycholic acid (DCA) or chenodeoxycholic acid (CDCA). Egr-1 was upregulated in response to bile acids, however there was no difference in the level of upregulation between wild type and FXR knockout hepatocytes. Consistent with these results, Egr-1 was upregulated to a similar extent in the livers of wild-type and FXR knockout mice after bile duct ligation. These data show that FXR is not required for bile acid-dependent up-regulation of Egr-1 in hepatocytes. Next primary mouse hepatocytes were isolated and pretreated with either AG1478, an EGFR inhibitor, or U0126, an inhibitor of MEK-1 before bile acid treatment. Pretreatment with both inhibitors prevented upregulation of Egr-1 after bile acid treatment, suggesting that activation of EGFR and the MAP kinase signaling cascade is required for upregulation of Egr-1 by bile acids. Taken together, these results suggest that inhibition of EGFR may be a novel target of therapy to prevent inflammatory liver injury in patients with cholestatic liver disease.

831 TIME-COURSE COMPARISON OF GENE EXPRESSION SIGNATURES OF XENOBIOTIC ACTIVATORS OF CAR AND PPARα IN MOUSE LIVER.

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Constitutive Androstane Receptor (CAR) and Peroxisome Proliferator-Activated Receptor(PPAR)α belong to a class of ligand-activated transcription factors called nuclear receptors and play an important role in xenobiotic metabolism. Upon activation, CAR and PPARα transactivate a plethora of genes involved in the biotransformation of xenobiotics. Phenobarbital (PB) and Wy-14,643 (WY) have been widely used as prototypical activators of CAR and PPARα, respectively. First, we examined CAR-mediated transcriptional effects of PB over a time course using microarrays. Male mice (C57BL/6J background) and corresponding Car-nulls were administered an acute dose of PB (100 mg/kg, i.g.) or vehicle and sacrificed at 24h. For sub-acute exposure, mice were given PB-containing (850 ppm) or control diet and sacrificed at 7 or 28d. Genes that changed significantly over the time course were identified using Extraction of Differential Gene Expression analysis, and significant biological processes were determined. In wild-type mice, a temporal biphasic (upregulated at 24h and downregulated at 28d) response in lipid, steroid, and cholesterol metabolism was observed in response to PB. No significant pathways were observed in Car-null mice. Next, to determine commonalities and differences in the temporal response to activators of two different nuclear receptors, we compared gene expression signatures from C57BL/6J male mice treated with PB or WY (50 mg/kg i.g., or 500 ppm in diet) for 24h, 7d, and 28d. Gene-by-gene comparison showed 168 genes in common between the treatments while 1369 genes were specific to PB and 1001 were specific to WY over the time-course. Similar pathways were affected by both compounds, but the regulation of these genes showed differences over time. This study establishes common fingerprints of exposure to activators of CAR and PPARα in rodent liver and demonstrates the changes that correlate with the presence or absence of a corresponding nuclear receptor. Supported by ES11391.

832 ACTIVATION OF THE ARYL HYDROCARBON RECEPTOR PROMOTES HEPATIC LIPOGENESIS.

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The AhR is well known as a ligand-dependent intracellular receptor that can stimulate gene transcription in response to xenobiotics, although, natural occurring ligands do exist. In this study, our findings suggest that AhR plays an endobiotic role by promoting hepatic lipid homeostasis. Activation of AhR by genetic (liver-specific CA-AhR transgene) or pharmacological (ligand with TCDD) activation markedly demonstrated hepatic lipid accumulation in animal models, showed by the oil red-o staining technique. The lipid accumulation in the transgenic and ligand-treated C57BL/6J mice was associated, by real-time PCR, with an increased expression of the free fatty acid transporter CD36 and the rate-limiting enzyme in triglyceride hydrolysis LPL. Expression of activated AhR in the livers of CA-AhR transgenic mice also resulted in increased hepatic triglyceride levels. Moreover, pharmacological activation of AhR in human hepatocytes showed the hepatic induction of CD36 and LPL. We propose that AhR plays an important role in hepatic lipid homeostasis by regulating the expression of CD36 and LPL, which are genes that facilitate the process of lipogenesis by the uptake and transport of fat molecules.

833 MECHANISMS OF HEPATOPROTECTION DURING LCA-INDUCED CHOLESTASIS: ROLE OF APOTOTIC SIGNALING.

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Activation of the constitutive androstane receptor (CAR) has been shown to protect the liver against bile acid-induced toxicity. CAR is involved in transcription of several genes relating to xenobiotic transport and metabolism, and may have a role in cell death by altering Bcl-2 protein expression. Hydrophilic bile acids associated with cytoprotection operate through various mechanisms, including apoptosis. To determine if hepatoprotection from bile acid-induced cholestasis involves a reduction in apoptotic cell death, C57BL/6 mice were pre-treated with phenobarbital (PB), oltipraz (OPZ), TCPOBOP (TC), and PCN for 3 days and then treated with lithocholic acid (LCA) (with continued inducer treatment) for 4 days to induce intrahepatic cholestasis. Histopathology revealed diffuse hepatocellular necrosis in the OPZ and LCA-only groups; while this pathology was absent in the PB, TC and PCN (protected) groups. Histologic protection correlated with liver enzymes as ALT was decreased in PB, TC and PCN pretreated groups compared to OPZ and LCA-only. Signs of apoptosis included increased activation of cleaved caspase 3 and decreased Mcl-1 activation in the OPZ and LCA-only groups, whereas these indicators of apoptosis were absent in the PB, TC and PCN pretreated mice. However, mRNA expression of pro- and anti-apoptotic genes did not correlate with the observed protection. Anti-apoptotic Mcl-1 expression was increased in the LCA and OPZ groups, whereas Bcl-xl was increased in the PB and OPZ groups. Pro-apoptotic expression of Bax was only increased in the PCN group. Although the expression of pro- and anti- apoptotic mediators in response to hepatoprotective pretreatment does not correlate with the observed decrease in apoptosis, alterations in mitochondrial membrane permeability may be a relevant pathway due to the increased hydrophilicity of metabolized bile acids in response to PB, TC, and PCN induction. (DK068039)

834 GENE EXPRESSION PROFILING OF XENOBIOTIC METABOLIZING ENZYMES (XMES) THROUGH THE LIFE STAGES OF THE MALE C57BL/6 MOUSE.

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In the presence of foreign compounds, metabolic homeostasis of the organism is maintained by the liver's ability to detoxify and eliminate these xenobiotics. This is accomplished, in part, by the expression of XMEs, which metabolize xenobiotics and determine whether exposure will result in toxicity. Some evidence indicates age may alter the ability of organisms to process xenobiotics, but the degree to which age affects hepatic metabolism is not known. This project was designed to examine the changes in XMEs from early to late life stages in male C57BL/6 mice. Gene expression profiles of XMEs were generated using Affymetrix Mouse 430 2.0 arrays for early (GD19, PD7, PD32, PD67) and older (2, 6, 12, 18, 24 months) life stages. Four animals per age group were profiled. Principal component analysis showed a clear age-dependent separation in expression profiles between GD19, PD7 and PD67 hepatic transcripts. Gene expression changes in XMEs were the most abundant at the early time points (GD19 and PD7) and 24 months. No significant gene changes were observed between 6 and 2 month old mice. We found 365, 286, and 96 XMEs significantly altered in the GD19, PD7, and PD32 when compared to PD67 respectively. We found 69, 94, and 130 XMEs significantly altered in the 12, 18, and 24 months versus 2 months comparison, respectively. qRT-PCR was performed to confirm altered expression. These data confirm age-dependent changes in XME gene expression in male C57BL/6 mice. This information will be used to adapt pharmacokinetic models to reflect age-dependent differences in xenobiotic metabolism. (This abstract does not necessarily reflect US EPA policy).

835 ONCOTIC NECROSIS AND APOPTOSIS MEDIATE LIVER INJURY IN RESPONSE TO SUPEROXIDE FORMATION *IN VIVO*.

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Reactive oxygen species are implicated in cell death of various liver disease processes. It has been shown that superoxide formation causes caspase-dependent apoptosis in primary cultured rat hepatocytes (Conde de la Rosa et al., J Hepatol. 44:918-29, 2006). To verify these findings in vivo, male Fisher rats were treated with 0.15 mmol/kg of the superoxide-generating herbicide diquat. The oxidant stress induced by diquat was confirmed by the massive increase of biliary glutathione disulfide excretion. Measurement of plasma ALT activities showed a gradual increase from 46 ± 4 U/L in controls to 955 ± 90 U/L at 6 h. H&E staining of liver sections revealed large areas of necrotic cells at 3 and 6 h. DNA strandbreaks were evaluated with the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay. Liver sections showed a slightly elevated number of TUNEL-positive cells with apoptotic morphology (cell shrinkage, chromatin condensation) at 3 and 6 h. However, there were clusters of TUNEL-positive cells, where the staining was predominantly cytosolic and the cells were swollen indicating oncotic necrosis. Measurement of small nuclear DNA fragments released into the cytosol with an anti-histone ELISA assay demonstrated a 40% increase over baseline. There was no significant increase in caspase-3 activities at any time between 0 and 6 h after diquat treatment. On the other hand, if animals were treated with galactosamine/endotoxin as positive control for apoptosis, caspase-3 activities were increased by 540%, the number of TUNEL-positive cells with apoptotic morphology was increased 52-fold and DNA fragmentation was enhanced 15-fold compared to controls. The data indicate that liver cell death initiated by superoxide formation in vivo is mediated predominantly by oncotic necrosis. The rate of apoptosis is elevated above baseline. However, the contribution of apoptotic cell death to the overall liver injury appears to be minor and of limited relevance for the pathophysiology.

836 ROLE OF APOPTOSIS-INDUCING FACTOR IN ACETAMINOPHEN-INDUCED LIVER CELL NECROSIS.

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DNA fragmentation is a hallmark of acetaminophen (APAP)-induced cell death. We showed recently the nuclear translocation of apoptosis-inducing factor (AIF), which correlated with DNA fragmentation, after APAP overdose (Bajt et al., Tox Sci 94: 217-225, 2006). When released from mitochondria, AIF combines with cyclophilin A and forms an active DNase. On the other hand, mitochondrial AIF is critical for oxidative phosphorylation and can function as superoxide-generating NADH oxidase. To address the hypothesis that AIF may be critical for APAP-induced cell death, male AIF-deficient mice (Hq) and wildtype animals were treated with 200 mg/kg APAP. Liver injury (plasma ALT activity, area of necrosis) and nuclear DNA damage (TUNEL assay and anti-histone ELISA) were evaluated at 6 h after APAP. In wildtype animals, APAP induced severe liver injury (plasma ALT activities: 8600 ± 1870 U/L and 61 ± 8% necrosis). The injury was accompanied by massive DNA strandbreaks in centrilobular hepatocytes and release of DNA fragments into the cytosol. In contrast, Hq mice had significantly less liver injury (ALT: 330 ± 130 U/L; necrosis: 4±2%) and minimal nuclear DNA damage at 6 h. Both wildtype and Hq mice had the same baseline levels of glutathione (GSH) and glutathione disulfide (GSSG), showed the same (75%) depletion of hepatic GSH 20 min after APAP and had a similar recovery of liver GSH levels at 6 h. However, hepatic levels of GSSG and immunohistochemical staining for nitrotyrosine protein adducts, which reflect a mitochondrial oxidant stress and peroxynitrite formation, resp., were only increased in APAP-treated wildtype animals and remained near baseline in Hq mice. These data indicate an absence of APAP-induced mitochondrial oxidant stress in Hq mice. Our data suggest that AIF has a dual function in APAP hepatotoxicity. AIF is important for the generation of reactive oxygen in mitochondria (NADH oxidase function) and, upon release from mitochondria, is involved in DNA fragmentation (DNase function).

837 ROLE OF TISSUE FACTOR AND PROTEASE ACTIVATED RECEPTOR-1 IN ACETAMINOPHEN-INDUCED HEPATOTOXICITY IN MICE.

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Acetaminophen (APAP) hepatotoxicity is associated with activation of the coagulation system in humans and mice. We have shown that inhibition of the coagulation protease thrombin reduces APAP-induced liver damage. However, the pathways involved in APAP-induced activation of coagulation are not completely understood. We tested the hypotheses that tissue factor (TF), the primary cellular initiator of blood coagulation, plays a role in APAP-induced coagulation and that the thrombin receptor, protease activated receptor-1 (PAR-1), contributes to APAP-induced liver injury. Fasted C57BL/6J mice were treated with APAP (400 mg/kg, ip) or saline, and liver and blood samples were collected 0.5 - 24 hrs later. Treatment with APAP resulted in a rapid and transient increase in liver procoagulant activity. This was followed by an increase in plasma thrombin levels, monitored with a thrombin-antithrombin (TAT) assay and hepatic fibrin deposition. To determine the role of TF in APAP-induced coagulation and liver injury, mice expressing low levels (i.e., 1% of wild type) of TF (low TF mice) were treated with APAP. The plasma concentration of TAT, hepatic fibrin deposition and hepatocellular injury were significantly reduced in the low TF mice compared to control mice 6 hours after APAP treatment. One mechanism by which thrombin could promote APAP-induced liver injury is by activating PAR-1 signaling. Importantly, PAR-1-deficient mice developed less injury than wild type mice 6 hours after APAP administration. Neither TF deficiency nor PAR-1 deficiency prevented the APAP-induced consumption of liver glutathione. These results suggest that APAP-induced TF activity generates thrombin that activates PAR-1 and contributes to APAP-induced hepatocellular injury.

838 EFFECT OF SAME TREATMENT GIVEN AFTER ACETAMINOPHEN (APAP) OVERDOSE ON HEPATIC TRANSMETHYLATION SUBSTRATES.

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Acetaminophen (APAP) is the leading cause of drug induced liver failure. Treatment of APAP overdose requires administration of n-acetylcysteine (NAC) within 1-6 hr after APAP exposure. S-adenosyl-L-methionine (SAMe) and S-adenosylhomocysteine (SAH) are substrates critical in hepatic transsulfuration reactions to form glutathione and transmethylation reactions of nucleic acids, proteins, phospholipids and nucleic acids. APAP hepatotoxicity may impact repair process involving SAMe and the transmethylation pathway. This study tested the hypothesis that APAP is associated with inhibition of the balance of SAMe and SAH as a function of time and that treatment with SAMe after APAP exposure will modulate these effects. A separate experiment further evaluated the effect of an equimolar dose of NAC administered 1 hr after APAP overdose on hepatic SAMe and SAH levels. Male C57BL/6 mice (16-22 grams) were treated with vehicle (VEH; water 15 ml/kg ip injection) or 250 mg/kg APAP (15 ml/kg, ip). SAMe (1.25 mmol/kg) and NAC (1.25 mmol/kg) were administered 1 hr after APAP injection (SAMe+APAP) and (NAC+APAP), resp. Hepatic tissue was collected 4 and 6 hr after APAP administration. SAMe and SAH levels were determined following extraction of tissue by HPLC analysis. SAMe treatment elevated hepatic SAMe levels. NAC did not alter hepatic SAMe or SAH levels. SAMe and SAH hepatic levels were decreased 4 hr after APAP administration when compared to VEH. Treatment with SAMe 1 hr after APAP overdose was sufficient to reverse the depletion in hepatic SAMe and SAH levels. An equimolar dose of NAC administered 1 hr after APAP also prevented APAP mediated decline in SAMe and SAH. These results indicate that APAP induces a decline in hepatic SAMe levels which can impact transmethylation and transsulfuration pathways. Administration of SAMe or NAC 1 hr after APAP overdose successfully prevented the decline in hepatic transmethylation substrates. (Supported by NIH Grant 5P20RR016477 to the West Virginia IDeA Network for Biomedical Research Excellence).

839 CAVEOLIN-1 IS REQUIRED FOR HIGH MOBILITY GROUP BOX PROTEIN-1 (HMGB1)-INDUCED MACROPHAGE ACTIVATION AND NITRIC OXIDE PRODUCTION DURING ACETAMINOPHEN-INDUCED HEPATOTOXICITY.

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Toxic doses of acetaminophen (AA) cause centrilobular hepatic necrosis. We have previously demonstrated that macrophages and inflammatory mediators including nitric oxide generated via inducible nitric oxide synthase (iNOS) play key roles in AA-induced hepatotoxicity. Mechanisms regulating macrophage activation and the release of nitric oxide were analyzed. HMGB1 is a chromatin binding protein released from necrotic cells that functions as a mediator of inflammation following injury. HMGB1 exerts its actions by binding to receptor for advanced glycation end products (RAGE) on macrophages which leads to release of inflammatory mediators. We postulated that the HMGB1 pathway is important in regulating macrophage activation and mediator release during AA-induced hepatotoxicity. Treatment of WT mice with AA (300 mg/kg, i.p.) resulted in increased expression of iNOS in the liver and nitrotyrosine staining in centrilobular regions. This was associated with a marked decrease in expression of the membrane scaffolding protein

caveolin-1 (Cav-1). To analyze the role of Cav-1 in HMGB1 signaling we used Cav-1-/- mice which are resistant to AA-induced hepatotoxicity. In WT mice HMGB1 staining was predominantly localized in hepatocyte nuclei. Treatment of mice with AA resulted in a time-dependent loss of HMGB1 from hepatocytes and an increase in staining of liver macrophages which was first evident within 3 h and coincided with the development of hepatic necrosis. In contrast, APAP had no effect on HMGB1 expression in Cav-1-/- mice. Moreover, nitrotyrosine staining in the livers of the Cav-1-/- mice was significantly reduced. These data, together with reports that mice treated with anti-RAGE antibody are protected from APAP-induced hepatotoxicity suggest that HMGB1 plays an important role in macrophage release of cytotoxic mediators such as nitric oxide. Moreover, Cav-1 is required for HMGB1 mediated activation of macrophages and the release of inflammatory mediators. Supported by NIH GM034310, ES004738, ES005022

840 AUTOPROTECTION AGAINST ACETAMINOPHEN HEPATOTOXICITY: CLUES FROM A GENE EXPRESSION PROFILE ANALYSIS.

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Pretreatment of mice with a low dose of acetaminophen (APAP) results in resistance to a subsequent, more toxic dose. In the present study this model of APAP autoprotection was employed. Male C57BL/6J mice were treated with APAP (400 mg/kg, i.p.) and were then challenged 48 hrs later with a second, higher dose of APAP (600 mg/kg) and sacrificed 4 or 24 hrs post second dose. Mice pretreated with APAP displayed less hepatotoxicity as indicated by plasma ALT activity and histopathology compared to mice receiving only a toxic dose. Total hepatic RNA was isolated and hybridized to Affymetrix Mouse Genome MU430_2 GeneChips. Statistically significant genes were determined using pair-wise t-tests ($p<0.05$, fold change> +/- 2) to identify genes that may be related specifically to the toxic dose or to autoprotection. Removal of genes differentially expressed in the groups that received only the challenge dose resulted in a subset of 611 genes. Detailed pathway analysis and literature review of these genes revealed a smaller number of genes showing mechanistic potential for involvement in APAP autoprotection. Up-regulation of genes such as galectin-3 and glycoprotein transmembrane NMB provides evidence towards the suppression of toxic effects as well as the promotion of hepatocyte proliferation, while flavin containing monooxygenase 3 suggests that an alternative metabolic pathway may also play a role. Elevated levels of alpha fetoprotein may indicate increased differentiation of oval hepatic progenitor cells within the liver. All of these genes portray functions that have the potential to contribute to APAP autoprotection. These genes will be further studied in future experiments to determine the extent of their roles in autoprotection against APAP hepatotoxicity. This work was supported by NIH Grant DK069557 and Pfizer Inc.

841 MECHANISMS OF PROTECTION BY SAPONINS DERIVED FROM ROOTS OF PLATYCODON GRANDIFLORUM AGAINST CARBON TETRACHLORIDE-INDUCED LIVER DAMAGE IN MICE.

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The purpose of this study was to investigate the protective effects of the saponins derived from roots of Platycodon grandiflorum (CKS)on carbon tetrachoride (CCl4)-induced hepatotoxicities and the possible protective in mice. CKS pretreatment prior to the administration of CCl4 significantly prevented the increase in ALT and AST activities and hepatic lipid peroxidation formation. In addition, CKS essentially prevented CCl4-induced apoptosis and necrosis, as indicated by a liver histopathologic study and DNA laddering. Pretreatment with CKS markedly decreased CCl4-induced Fas/FasL protein expression levels and in turn attenuated CCl4-induced caspase-3, -8 activities in mouse livers. Additionally, CKS pretreatment protected the CCl4-induced depletion of hepatic glutathione (GSH) levels. The effect of CKS on cytochrome P450 (CYP) 2E1, the major isozyme involved in CCl4 bioactivation, was investigated. Treatment with CKS resulted in a significant decrease in the CYP2E1-dependent hydroxylation of aniline in a dose-dependent manner. In addition, CKS exhibited anti-oxidant effects on FeCl2-ascorbate induced lipid peroxidation in mouse liver homogenates, and on superoxide radical scavenging activity. These findings suggest that the protective effects of CKS against CCl4-induced acute liver injury possibly involve mechanisms related to its ability to block CYP2El-mediated CCl4 bioactivation and its free radical scavenging effects, and that is also protects against Fas/FasL pathway mediated apoptosis.

842 METHYL-DONOR ENRICHMENT ATTENUATES ALCOHOL-INDUCED LIVER INJURY:MECHANISMS OF PROTECTIVE ACTION.

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Increasing evidence suggests that chronic alcohol abuse leads to abnormal hepatic methionine metabolism resulting in decreased S-adenosylmethionine (SAM) concentrations contributing to the development of alcohol-induced liver injury (ALI). The mitigation of disease severity in animal models of ALI has been achieved through dietary supplementation of methionine cycle metabolites including SAM and betaine, agents that are thought to act by restoring methyltransferase reactions. However, the effects of these dietary supplements on alcohol metabolism are unclear. Thus, C57BL/6J mice were administered a high fat diet (HFD), or methyl-donor (MD) enriched HFD (MDHFD) with or without alcohol (up to 27 g/kg/day) for 4 wks using the intragastric Tsukamoto-French protocol. Significant attenuation of hepatic injury was achieved in mice administered MDHFD compared to HFD, an effect accompanied by elevations in methionine and SAM as well as a reduction in GSSG:GSH in liver. Blood and liver alcohol concentrations were not different between groups at sacrifice; however, mean urine alcohol concentrations were reduced by 50% with MDHFD. When acute dose of ethanol (2 g/kg) was administered to mice on control or MD-enriched diets, blood alcohol elevations and stomach catalase activity were similar suggesting that protective action of MD diet is not due to the effect on first pass metabolism of alcohol in the stomach. Therefore, we suggest that methyl-donor enrichment restores redox state in the liver, increases NAD supply and diminishes fluctuations in the activity of alcohol dehydrogenase. Understanding the mechanistic role of methionine intermediaries may help in development of effective and safe therapies for alcoholic liver disease.

843 PROTECTIVE EFFECTS OF *IN VIVO* S-ADENSOYLMETHIONINE ADMINISTRATION ON CHRONIC ALCOHOL INDUCED MITOCHONDRIAL DYSFUNCTION.

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Some of the earliest pathologic changes induced by chronic alcohol consumption occur at the level of the mitochondrion in liver. Chronic alcohol increases sensitivity to apoptotic stimuli and induction of the mitochondrial permeability transition (MPT). Evidence indicates that hepato-protection by S-adenosylmethionine (SAM) may be mediated through effects on mitochondria however the targets of SAM are unknown. To determine the molecular mechanism of SAM hepato-protection, an animal model of alcoholic liver disease was used to explore the effect of SAM to attenuate alcohol dependent alterations in mitochondrial function. For this, rats were pair-fed control or ethanol-containing liquid diets +/- SAM and calcium induction of the MPT was monitored. Induction of the MPT was monitored by measuring the rate of mitochondrial swelling in the presence of increasing concentrations of calcium (50-1000 micromolar) in a physiological relevant phosphate buffer. Liver mitochondria from rats fed alcohol chronically were more sensitive to calcium induced MPT pore opening than controls. Interestingly, in vivo SAM supplementation decreased the rate of induction of the MPT in ethanol-fed mitochondria by 15%. In all groups, induction of the MPT was blocked by cyclosporin A. Oxygen consumption was measured in the presence of oxidizable substrates and ADP to further assess mitochondrial function. Mitochondrial state 3 respiration (ADP-dependent) and the respiratory control ratio were decreased in animals fed ethanol compared to controls, whereas respiratory function was preserved in alcohol-fed animals co-administered SAM. In conclusion, these results suggest that SAM supplementation decreases the rate of induction of the MPT and attenuates alcohol-dependent decrease in mitochondrial respiration. Therefore, the ability of SAM to preserve mitochondrial function after chronic alcohol exposure could serve as a therapeutic strategy to prevent alcohol toxicity and thereby slow the progression of alcoholic liver disease.

844 CYTOKINE AND CHEMOKINE EXPRESSION ASSOCIATED WITH STEATOHEPATITIS AND HEPATOCYTE PROLIFERATION IN RATS FED ETHANOL *VIA* TOTAL ENTERAL NUTRITION.

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To determine the association of altered immune responses with development of alcoholic liver pathology, male Sprague-Dawley rats were intragastrically fed low carbohydrate-containing ethanol (EtOH) diets via total enteral nutrition for up to 49 d. Induction of EtOH metabolism and appearance of steatosis preceded development of oxidative stress, inflammation, and cell death. A transitory peak of tumor necrosis factor alpha (TNF) and interferon gamma (IFN) was observed at 14 d followed by reduced expression of TNF, IFN and IL-12 accompanied by reduced expression of the Th1 regulators T-bet and STAT4. After 35-49 d of EtOH, at a time when hepatocyte proliferation was stimulated a second peak of TNF occurred. The Th2 cytokine IL-4 remained suppressed throughout the study and was accompanied by reductions in the Th2 regulator GATA3. There was no temporal effect of EtOH on expression of IL-6 or TGF beta. IL-5 and IL-13 mRNA were undetectable. Chemokine CXCL-2 expression increased progressively up to 35 d and preceded the appearance of inflammatory infiltrates. These data suggest that steatosis, increased ethanol metabolism, a transient induction of the innate immune response and suppression of Th2 responses were acute consequences of ethanol treatment and were followed by suppression of Th1 responses. However, necrosis, apoptosis and a late peak of TNF only occurred after 6-7 weeks of ethanol, coincided with the appearance of inflammatory infiltrates and were associated with stimulation of hepatocyte proliferation.

845 THE INFLUENCE OF SEX STEROIDS IN ALCOHOLIC LIVER INFLAMMATION.

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Alcohol liver disease (ALD) is more severe in females than males. Proinflammatory cytokine release from Kupffer cells during inflammation is associated with the pathogenesis of ALD, and female chronically ethanol fed rats express cytokine mediators differently than male counterparts. Differences in IL-6 receptor function in females may play a role in the clinical observations of more severe ALD in females. In this study, the effects of sex steroid exposure on the expression and function of IL-6 receptor in ethanol fed rats were examined. Normal male and female or ovarectomized Wistar rats were fed liquid diet with and without ethanol for up to four weeks. Ovarectomized rats were additionally implanted with osmotic pumps containing either testosterone or estrogen. Ethanol consumption significantly increased in IL-6 receptor mRNA and protein expression and NFkB activation in female and estrogen treated rats. However C/EBPβ, IkB expression was higher while NFkB activation was lower in male and testosterone treated rats, indicating increased IL-6 signaling, and decreased NFkB signaling respectively. Our data suggest that sex steroids play a role in IL-6R expression and function and that testosterone may be protective in ALD.

846 OSTEOPONTIN –MEDIATED B1 AND B2 INTEGRIN SIGNALING: A MECHANISM FOR HIGHER HEPATIC NEUTROPHIL INFILTRATION AND LIVER INJURY IN FEMALE ALCOHOLIC LIVER DISEASE.

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Females are known to be more susceptible to alcoholic liver disease (ALD), but the precise mechanism behind the increased susceptibility of females is not well understood. Previous studies from our laboratory have shown that osteopontin (OPN) mediates hepatic neutrophil infiltration making females more susceptible to alcoholic steatohepatitis (ASH). The objective of this study was to investigate the patho-mechanism by which OPN attracts the neutrophils into the liver. We hypothesized that higher OPN-mediated hepatic neutrophilic infiltration is a result of SLAYGLR signaling of OPN through its receptor α9β1 (VLA9) and α4β1 (VLA4) integrins on neutrophils. Compared to the males, females in the ASH group showed higher expression of α4β1 and α9β1 protein and mRNA. A significant decrease in the expression of these integrins was observed in animals treated with neutralizing OPN antibody, wherein the hepatic neutrophil infiltration decreased to almost 50% compared to the untreated group. Immunoprecipitation experiments suggested the binding of OPN to α4β1 and α9β1 integrins. OPN-mediated neutrophilic infiltration was also confirmed using Boyden chamber assays. Antibodies directed against α4, β1 integrins and SLAYGLR sequence was also found to significantly inhibit neutrophilic migration in vitro. Furthermore, flow cytometry results revealed OPN mediated up-regulation of neutrophil CD11b/CD18 (Mac1; β2 integrin) in the blood. In addition to hepatobiliary OPN, neutrophils isolated from animals in ASH group, were also found to be positive for OPN. In conclusion, these data suggest that that higher hepatobiliary expression of OPN is the likely reason for higher hepatic neutrophil chemokinesis in the female ASH which appears to be mediated through both α4β1 and α9β1 integrin signaling (supported by NIH-AA016316 and Graduate student fellowship- Novartis award).

847 COEXPOSURE OF MICE TO TROVAFLOXACIN AND LIPOPOLYSACCHARIDE RESULTS IN A UNIQUE GENE EXPRESSION PROFILE AND VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF)-DEPENDENT LIVER INJURY.

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Trovafloxacin (TVX) is a fluoroquinolone antibiotic which has caused idiosyncratic hepatotoxicity in a small fraction of people. Animal models predictive of drugs that cause idiosyncratic adverse drug reactions (IADRs) are lacking in preclinical safety testing. Previously we showed that a modest inflammatory stress induced by lipopolysaccharide (LPS) renders mice sensitive to nonhepatotoxic doses of TVX. In contrast, levofloxacin (LVX), a fluoroquinolone antibiotic that has not been associated with IADRs in humans, does not cause hepatotoxicity in mice cotreated with LPS. TVX and LPS coexposure induced a significant increase in plasma ALT activity (5062 +/- 1263 U/L, p<.001) and caused midzonal coagulative necrosis in the livers of mice. Hierarchical cluster analysis of hepatic gene expression data from mice taken at 3 h after LPS, a time before the onset of liver injury, revealed treatment-related clustering. This unique clustering was corroborated by principal component analysis. A pathway highly affected by TVX/LPS treatment was hypoxia-inducible factor signaling. One gene in this pathway which has been associated with liver injury is vascular endothelial growth factor (VEGF). TVX prolonged the early increase in plasma VEGF caused by LPS (2-fold, p<.001). Inhibition of VEGF activity by VEGF antiserum protected mice from TVX/LPS-induced liver injury, as reflected by both plasma ALT activity (ALT decrease from a mean of 3426 U/L to 1175 U/L, p =.03) and liver histopathology. In summary, TVX/LPS coexposure induced unique gene expression at a time before the onset of liver injury in mice. VEGF appears to play a critical role in the progression of TVX/LPS-induced liver injury. (Supported by NIH grants DK061315 and GM075865.)

848 HEMOSTATIC SYSTEM ACTIVATION CONTRIBUTES TO HEPATOTOXICITY IN MICE TREATED WITH TROVAFLOXACIN AND LIPOPOLYSACCHARIDE.

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Idiosyncratic adverse drug reactions (IADRs) represent a significant problem for human health. The mechanisms of IADRs are not well understood, but inflammatory stress might play a role in IADRs by increasing susceptibility to toxicity. Trovafloxacin (TVX) is a fluoroquinolone antibiotic linked clinically to the occurrence of hepatotoxic IADRs. Treatment of mice with a nontoxic dose of TVX followed 3 h later by a noninjurious dose of the potent inflammagen, bacterial lipopolysaccharide (LPS), led to significant hepatotoxicity. Using this animal model of IADRs, we tested the hypothesis that activation of the hemostatic system occurs selectively in mice cotreated with TVX and LPS and contributes to the development of liver injury. At a time near the onset of hepatotoxicity (4.5 h following LPS treatment), cotreated mice had decreased concentrations of plasma fibrinogen and increased concentration of thrombin-antithrombin dimers compared to those mice treated with either agent alone. These results suggest that the coagulation system was activated in cotreated mice. Hepatic fibrin deposition was also greater in cotreated mice. In addition, these mice had increased concentrations of plasminogen activator inhibitor-1 in the plasma, consistent with suppression of the fibrinolytic system. Plasma alanine aminotransferase measured 15 h after administration of LPS and liver histopathology indicated hepatocellular damage in mice cotreated with TVX and LPS. Treatment with anticoagulant heparin 2 h after LPS decreased hepatic fibrin deposition and significantly reduced hepatotoxicity in cotreated mice. These results suggest that activation of the hemostatic system contributes to the progression of liver injury caused by TVX and LPS cotreatment in this model of IADRs. (Supported by NIH grant DK 061315.)

849 ROLE OF P38 MAPK KINASE IN TNF-α PRODUCTION AND LIVER INJURY IN HEPATOTOXIC INTERACTION OF LIPOPOLYSACCHARIDE AND RANITIDINE.

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Cotreatment with nontoxic doses of lipopolysaccharide (LPS) and ranitidine (RAN) causes liver injury in rats. Gene expression analysis suggested possible involvement of P38 MAPK pathway in the liver injury. Previous studies showed RAN augmented serum TNF-α production, hepatic neutrophil activation after LPS treatment. Both TNF-α and neutrophils are crucial for the pathogenesis. We tested the hypothesis that P38 is necessary for TNF-α production, neutrophil activation and subsequent liver injury caused by LPS/RAN cotreatment. LPS/RAN cotreatment caused robust P38 activation compared with treatment with LPS only. SB239063, a P38 inhibitor, reduced LPS/RAN-induced hepatotoxicity. This inhibitor also reduced plasma PAI-1 and thrombin-antithrombin dimer concentration and neutrophil activation in livers. The P38 inhibitor reduced serum TNF-α to the same level as LPS only treatment. However, the inhibitor did not reduce TNF-α mRNA in liver, suggesting a post-transcriptional mode of action. This might occur through TNF converting enzyme (TACE), which cleaves pro-TNF into its active form. Indeed, a TACE inhibitor administered just before RAN treatment reduced serum TNF-α protein. The TACE inhibitor also reduced liver injury caused by LPS/RAN cotreatment. In summary, RAN enhanced TNF-α production after LPS treatment through augmented P38 activation, and this appears to occur through the activation of TACE. (Supported by NIH grant DK061315)

850 DETERMINANTS OF SENSITIVITY TO HALOTHANE-INDUCED HEPATOTOXICITY IN MICE.

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The once widely used anesthetic halothane (2-bromo-2-chloro-1, 1, 1-trifluoroethane) induces a mild hepatitis in 20% of patients and idiosyncratic fulminate liver failure in approximately 1 in 30,000. The mechanism of the severe form of halothane hepatic injury is not entirely understood. We examined several factors for their ability to influence sensitivity of mice to halothane-induced liver injury. To study the influence of gender, male and female Balb/C mice were treated with halothane (15-45 mmol/kg), and liver injury was assessed 24 hrs later by the activity of serum alanine aminotransferase (ALT) and liver histopathology. In both males and females, the injury was dose-dependent. The female maximal ALT response (10,000 U/L) was greater than that in males and corresponds to severe hepatocellular injury. Female mice also developed liver injury at smaller halothane doses than males. Livers from both male and female Balb/C mice had centrilobular necrosis and accumulation of neutrophils consistent with the appearance of postmortem histological changes in patients who died from halothane-induced liver failure (Wills, EJ et. al, 1978). Fasting female Balb/C mice for 15 hr increased the sensitivity to halothane-induced hepatic injury. To test the possibility that a concurrent inflammatory episode could sensitize a mouse to halothane-induce hepatocellular injury, male Balb/C mice were given a mildly hepatotoxic dose of the halothane (15 mmol/kg) followed by a nonhepatotoxic dose of bacterial lipopolysaccharide (LPS; $5x10^6$ EU/kg), and serum ALT activity was assessed 24 hrs later. The cotreated animals had a 10-fold increase in serum ALT activity relative to mice treated with either agent alone. These results suggest that multiple intersecting determinants of sensitivity including gender, fasting, and inflammatory stress can result in severe liver injury from halothane exposure. (Supported by NIH grant GM075865.)

851 POSSIBLE ROLE OF OSTEOPONTIN IN TH1 IMMUNE RESPONSE AND LYMPHOCYTIC INFILTRATION DURING NON-ALCOHOLIC STEATOHEPATITIS IN A DIETARY MURINE MODEL.

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Non-alcoholic steatohepatitis (NASH) is becoming increasingly common in the developed world population. It is predicted that by the year 2025 greater than 25 million Americans may have NASH- related disease. However the precise mechanism for the occurrence of NASH is not clearly understood. The objective of the present study was to investigate the role of osteopontin (OPN) in the alterations of cytokines profile during the pathogenesis of NASH. A nutritional mice model (C57BL6 male) of NASH was developed, in which they were fed methionine choline deficient diet (MCD; Fat 10%) and controls received methionine choline sufficient diet for 6 weeks. MCD feeding resulted in a trend towards decrease in body weight compared to controls, although general health condition was similar in both groups. Significantly higher micro and macro vesicular fat droplets were found in MCD feeding mice as compared to their controls. In addition multifocal lympho-histiocytic infiltrates were identified, and T cell infiltrations were confirmed by CD3 staining by immunohistochemistry. Th1, Th2 and Th17 cytokine mRNA profiles evaluated by real time PCR showed increased Th1 (TNF-a, IFN-g, IL-12p40 and decreased Th2 (IL-4 and IL-10) cytokine profile. Interestingly, IL-17A and IL-17F mRNA levels were both elevated in the MCD group as compared to the controls, suggesting that Th17 response was also favored in addition to the Th1 response. OPN mRNA and protein expression were significantly higher in the MCD group compared to the controls. Studies underway are designed to test the precise effects of OPN in mediating Th1 polarization during lympho-histiocytic infiltration in NASH model. To conclude there appears to be a significant correlation between OPN induction and Th1 polarization during hepatic lymphocyte infiltration in the nutritional oxidative stress NASH model.

852 EFFECTS OF TCDD ON LIVER LIPID METABOLISM – MOUSE *VERSUS* RAT.

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TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) elicits a broad range of tissue-, sex-, and species-specific effects. This study compares the hepatic response to a single oral dose of TCDD in immature ovariectomized (i.o.) C57BL/6 mice (30 μg/kg) and i.o. Sprague-Dawley rats (10 μg/kg). Animals were sacrificed at 72, 120, or 168 h postdose and hepatic lipid extracts were analyzed by quantitative proton-decoupled 13C and 31P NMR (14.1 T). TCDD-induced effects on hepatic lipids (relative to vehicle controls) were significantly more pronounced in mice, where lipid content increased by 55% at 72 h and remained elevated at 168 h ($p \leq 0.05$). Rats showed a slight, but not significant, increase in lipid content; the only significant effect being a 41% increase in n6-fatty acids (FA) at 120 h (39.4 ± 2.9 vs 28.0 ± 2.6 μmol/g liver, $p \leq 0.05$). In mice, TCDD significantly ($p \leq 0.05$) increased triacylglycerol (TAG), cholesterol, n3-FA, and n6-FA at 72 h (41-65%). By 168 h, n3-FA returned to control levels but TAG and n6-FA remained elevated; cholesterol was also 45% > control at 168 h, but highly variable and non-significant. Rats showed no significant changes in any phospholipids, whereas in mice (1) phosphatidylethanolamine and sphingomyelin increased 44% and 67%, respectively, at 72 h and returned to control levels by 120 h; (2) lysophosphatidylcholine increased 60% at 72 h and by 2-fold at 168 h; and (3) phosphatidylserine and phosphatidylinositol increased 3-fold and 2-fold, respectively, at 168 h ($p \leq 0.05$). Principal component analysis of 13C spectra corroborate these findings showing separation between control vs. treated groups in mice, but not rats. These species-specific changes in lipids are consistent with species-specific differential gene expression (Toxicol Sci 85, 1048 (2005)), suggesting TCDD elicits different effects in the rat compared to the mouse. Funded by RO1 ES013927.

853 DIOXIN ALTERS INFLAMMATORY RESPONSES TO LIPOPOLYSACCHARIDE.

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Inflammation is a complex process mediated by several cell types and soluble mediators. Administration of bacterial lipopolysaccharide (LPS) to experimental animals results in inflammation and consequent tissue injury, including hepatotoxicity. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) has well characterized effects on specific immune responses, but the effects on the innate immune system are less understood. Accordingly, we evaluated LPS-induced inflammation in C57BL/6J female mice exposed to TCDD. Mice were treated by oral gavage with 30 μg/kg TCDD or vehicle once, and four days later they received LPS (5x10E5 EU/kg i.p.). Inflammatory mediators as well as the liver injury marker, alanine aminotransferase (ALT), were measured in plasma, and liver histology was evaluated. TCDD-treated animals had greater plasma ALT activity when treated with LPS, but the effect was mild. Few changes in liver histopathology were observed, and they included greater steatosis and less neutrophil infiltration in LPS/TCDD-treated mice compared to mice treated with LPS alone. Dose-response curves for plasma concentration of interleukin (IL)-6 suggested that dioxin increased the potency of LPS to cause release of this cytokine but not the efficacy. Plasma concentrations of IL-10, IL-12, gamma interferon, monocyte chemoattractant protein-1, and tumor necrosis factor alpha were greater in mice treated with TCDD and LPS compared to those treated with either agent alone. These results suggest that TCDD changes the inflammatory cytokine profile induced by LPS and that LPS enhances the hepatic steatotic response to TCDD (Supported by ES004911).

854 THE CONTRIBUTION OF PPAR-ALPHA TO HEPATIC RESPONSES IN A MOUSE MODEL OF INFLAMMATION-DIOXIN INTERACTION.

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The high prevalence of inflammatory conditions has raised interest in interactions between environmental pollutants and inflammation that might contribute to disease. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is an environmental toxicant that activates the aryl hydrocarbon receptor (AhR). A typical response to TCDD is hepatic steatosis. Lipid accumulation arises from alterations in fatty acid metabolism, a process that is regulated by peroxisome proliferator-activated receptor-alpha (PPAR-a). We tested the hypothesis that the response to TCDD in a murine model of acute inflammation induced by lipopolysaccharide-galactosamine (LG) is modulated by PPAR-a. Female C57BL/6 mice were treated orally with vehicle or the PPAR-a agonist, fenofibrate, for 13 days and then dosed with vehicle or 30 μg/kg TCDD (p.o.). Four days later, the animals received an ip injection of LG (5x10E5 EU/kg/500 mg/kg) or saline. After fours hours, animals were euthanized, and blood and liver samples were collected for analysis. Steatosis was detected by staining liver sections with oil red O. No staining was detected in livers of animals treated with either vehicle or LG alone. TCDD induced hepatic lipid deposition, and cotreatment with LG enhanced this response. Irrespective of treatment with LG, TCDD-induced steatosis was blocked by fenofibrate. Livers from mice exposed to TCDD and LG had fewer cells undergoing apoptosis and fewer hepatic neutrophils but greater plasma IL-6 concentration when compared to those treated with LG alone. Treatment with fenofibrate reversed these effects of TCDD on the response to LG. Fenofibrate abrogated protein expression of IKB-a, suggesting crosstalk between PPAR-a and NFkB signaling pathways as modulators of the AhR-dependent response. These data suggest that PPAR-a activation counteracts changes in lipid metabolism induced by TCDD and interferes with TCDD-induced modulation of the inflammatory response (Supported by ES004911).

855 PERFLUOROOCTANESULFONATE CONCENTRATIONS IN ADULT RAT LIVERS AFTER FIVE ORAL DOSES – COMPARISON BETWEEN LOBES.

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Perfluorooctanesulfonate (PFOS), a persistent and bioaccumulative compound, has been reported to distribute in serum and liver. Even though liver is comprised of several lobes and weighs approximately 10 – 15 grams in adult rats, advances in analytical techniques require only a small portion of the liver (i.e., 0.2 gram) to be extracted when analyzing for PFOS. The purpose of this study was to evaluate whether there was a difference in PFOS concentrations between different liver lobes in rats after receiving PFOS treatments.
Adult male SD rats were given 5 mg/kg PFOS potassium salt (N=5) or vehicle (0.5% Tween 20, N=2) via oral gavage for 5 days to establish a steady state in liver. The rats were sacrificed after last dose and sections of each liver lobe were dissected, weighed, and flash frozen pending analyses for PFOS via LC/MS-MS. The results indicated that there was no difference in PFOS concentrations among the liver lobes, suggesting that PFOS is homogeneously distributed in rat livers.

856 HEPATIC GENE EXPRESSION ANALYSES REVEAL ALTERATIONS IN CHOLESTEROL METABOLISM IN TR- RATS.

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The Abcc2 gene encodes multidrug resistance protein 2 (Mrp2), an ATP-binding cassette transporter expressed on the canalicular membrane of hepatocytes, which plays a critical role in organic anion efflux. TR- rats contain an inherited single nucleotide deletion within the Abcc2 coding sequence that results in insertion of an inappropriate stop codon, preventing expression of the transcript. Thus, TR- rats provide a useful model for studying the disposition and biliary excretion of xenobiotics. Hepatic transcriptional profiles, generated to assess global constitutive gene expression, indicated that 1,119 genes (fold change ≥ 1.5, $p \leq 0.01$) were differentially expressed in male TR- rats relative to wildtype Wistar rats. As expected, it was confirmed that TR- rats compensate for Mrp2-deficiency with increased hepatic expression of Mrp3. However, there was clear evidence of altered expression of genes involved in cholesterol biosynthesis and utilization. Specifically, Abca1 levels were decreased 4.4-fold, while the biliary cholesterol transporters Abcg5/8 were unchanged. Genes involved in cholesterol biosynthesis including acetoacetyl-CoA thiolase 1, HMG-CoA reductase, mevalonate kinase and lanosterol synthase (Lss) were significantly upregulated 2.1-, 3.5-, 2.8- and 33-fold, respectively, while Cyp7a1, the rate-limiting enzyme of bile acid biosynthesis was downregulated 2.7-fold. Lss, which catalyzes the cyclization of 2,3-monoexpoxysqualene to form lanosterol, was the most significantly upregulated gene in TR- rat liver. Transcriptional changes in cholesterol metabolism were not accompanied by detectable differences in hepatic or biliary cholesterol content. No changes in serum total cholesterol or total bile acids were noted, although serum LDL levels were 50% lower in the TR- rat than in the Wistar. These data suggest there is an intrinsic perturbation in cholesterol homeostasis in TR- rats that although not clinically significant in the naïve animal, may affect responses to xenobiotic exposure.

857 HEPATIC ALT 1 & 2 PROTEINS ARE DIFFERENTIALLY EXPRESSED FOLLOWING DEXAMETHASONE TREATMENT IN MICE.

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Sponsor: M. Lawton.

ALT is a marker of hepatotoxicity, but is also important in gluconeogenesis and thus may be an indicator of metabolic adaptation. The purpose of this study was to determine if ALT 1 or ALT 2 isoenzymes respond differently to dexamethasone (DEX) which increases glycogen synthesis and induces gluconeogenesis. Mice (n=4-5/group) were treated with DEX (25 & 75 mg/kg) once a day IP for 1 to 3 days and compared to controls (C) to determine if ALT 1 and ALT 2 would increase in response to a gluconeogenic stimulus. Hepatic glycogen (mg/mg protein) peaked at 24 hours (C=23.7 ± 16.3, DEX 25=576.0* ± 57.0, DEX 75=550.8* ± 27.8) [* = statistically significant at $p < 0.05$] and was still elevated at 72 hours (C=13.1 ± 14.5, DEX 25=246.9* ± 152.9, DEX 75=242.7* ± 33.3). Hepatic ALT levels (U/mg of protein) did not increase at 24 hours (C=994 ± 44, DEX 25=1096 ± 122, DEX 75=1039 ± 206) but was increased at 72 hours at 25 and 75 mg/kg (C=822 ± 68, DEX 25=1064* ± 64, DEX 75=1286* ± 64). Hepatic ALT 2 protein expression, as determined by quantitative Western blot, increased at 24 hours (C=100.0 ± 12.8, DEX 25=161.7* ± 18.0, DEX 75=173.1* ± 24.8) and at 72 hours (C=100 ± 44.4, DEX 25=232.3* ± 20.3, DEX 75=304.9* ± 35.1). Hepatic ALT 1 protein levels did not increase at 24 (C=100 ± 9.1, DEX 25=89.1 ± 36.7, DEX 75=94.3 ± 24.7) or 72 hours (C=100 ± 13.4, DEX 25=80.9 ± 6.4, DEX 75=93.4 ± 69.3). Serum ALT activity (U/L) was increased at 24 hours (C=66 ± 17, DEX 25=86 ± 18, DEX 75=136* ± 29) but not at 72 hours (C=116 ± 43, DEX 25=160 ± 40, DEX 75=131 ± 13). This increase in ALT activity at 24 hours was possibly due to an increase in ALT 2 since hepatic ALT 2 protein levels increased and hepatic ALT 1 did not. There was no concurrent increase in serum GDH, AST or ALP at either time point or no histological evidence of hepatic necrosis. This study suggests that increased hepatic ALT protein expression may contribute to elevated serum ALT activity without overt liver injury.

858 ATYPICAL HEPATOTOXICITY CAUSED BY PHOSPHOPHIORATE OLIGONUCLEOTIDE ISIS-147420 IS MEDIATED BY AN INTERFERON TYPE I RESPONSE.

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An antisense oligonucleotide (ASO), ISIS-147420, containing 2'-O-methoxyethylribose (MOE) modifications was found to cause profound hepatotoxicity as measured by serum transaminases, organ weights and body weights that was atypical of this class of oligonucleotides. Other antisense oligonucleotides aimed at the same target (cpt1-α) showed no evidence of toxicity while maintaining their ability to reduce target mRNA. Thus the off-target effects were investigated. Following subcutaneous injection of ISIS-147420 and control non-hepatotoxic ISIS-141923 in mice, serum transaminases elevation was observed 72 hours post ISIS-147420 treatment. Histological observation revealed extensive apoptosis, necrosis, as well as monocytic infiltration at 72 hours but failed to reveal any significant pathological change at 24 or 48 hours. Whole genome gene expression profiling was performed on livers collected at 8, 24, 48 and 72 hours. A large number of interferon stimulated genes (ISGs) and genes associated with antiviral responses were significantly upregulated as early as 24 hours. Among the genes upregulated at 24 hours is Stat1 followed by interferon-β at 48 hours. Activation of Stat1 by phosphorylation at 48 hours was also demonstrated. Administration of ISIS-147420 to Stat1 deficient mice showed no evidence of hepatotoxicity (no changes in transaminases levels or morphological changes) up to 96 hours post treatment. Furthermore, upregulation of ISGs observed in the control strain was not seen in Stat1 deficient mice. The complete abrogation of hepatotoxicity in Stat1 deficient background points toward an activation of the innate immune response by ISIS-147420 and more specifically

toward an interferon mediated mechanism of hepatotoxicity. Previous studies have shown the toxicity occurred independently of TLR9 or Myd88 and may have a specific sequence motif that might cause ISIS-147420 to be mistaken as a pathogen-associated molecular pattern similar to viral RNA or DNA triggering an innate immune response ultimately resulting in severe hepatotoxicity.

859 INVESTIGATION OF THE DIFFERENTIAL HEPATIC CONCENTRATION OF AN ANTI-INFLAMMATORY COMPOUND AND ITS ASSOCIATION WITH PHOSPHOLIPIDOSIS IN RODENTS.

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Many marketed drugs induce phospholipidosis (PLD) in preclinical toxicity studies. In many cases the compounds belong to a family of molecules referred to as cationic amphiphilic drugs (CADs) which includes the agents amiodarone and fluoxetine. Possible mechanisms of drug-induced PLD include alteration of lipid handling by modification of the lipid structure (e.g. by binding), or inhibition of the phospholipase enzymes involved in phospholipid breakdown.
We investigated an AZ molecule from a series of anti-inflammatory agents that possess CAD-like properties of which some have been shown to induce PLD *in vivo*. We studied the disposition of this agent in the liver of rodents by the use of radiolabelled methods and imaging mass spectrometry (IMS), and applied mathematical modelling to understand the relative disposition between liver and plasma. Radiolabelled compound was administered to rats and mice as single or repeat doses. Blood and liver samples were analysed for total radiolabel content by liquid scintillation counting. Liver tissue was also analysed by microautoradiography. The results of these studies showed that at doses that produce hepatic PLD there is an increased concentration of the agent in the liver compared to plasma. Furthermore, PBPK modelling using measured tissue and plasma concentrations suggests that high doses of the agent are handled differently in the liver compared to doses that do not produce PLD. In separate studies, liver tissue was taken from animals that had been dosed with non-radiolabelled material and analysed by IMS. These studies showed that this methodology may be useful in spatially resolving drug related material in the liver. In conclusion, these experiments indicate high liver concentrations of this compound are required to produce PLD. Furthermore, liver load that is associated with PLD in rodents may not be reflected by circulating plasma levels.

860 EFFECTS OF SYNTHETIC ANDROGENS ON LIVER FUNCTION USING THE RABBIT AS A MODEL.

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The objective of this study was to develop an animal model to test new synthetic androgens for potential liver toxicity. Adult male NZW rabbits (n=3/group) were dosed daily po on days (d) 0-13 with various androgens including the positive control, 17α-methyltestosterone (MT), and the negative control, testosterone (T), in order to validate this model. Synthetic androgens tested were 7α-methyl-19-nortestosterone (MENT), 7α,11β-dimethyl-19-nortestosterone 17β-undecanoate (DMAU), and 11β-methyl-19-nortestosterone 17β-dodecylcarbonate (MNTDC). Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transpeptidase (GGT), and sorbitol dehydrogenase (SDH), on d 0 (prior to po dosing), 3, 7, 10 and 14, and clearance of iv injected bromsulfonphthalein (BSP) on d 0, 7 and 14 were determined. None of the rabbits exhibited clinical signs, and body weights were not suppressed. BSP was cleared from serum within 20 min on d 0. At 10 mg T/kg/d, a reduction in BSP retention was observed on d 7 and 14, but serum SDH and AST levels were slightly increased on d 14. At 10 mg MT/kg/d, serum BSP retention was increased on d 7 and 14, and AST, ALT, GGT, and SDH were all increased on d 7, 10, and 14. DMAU and MENT at 10 mg/kg/d, increased BSP retention on d 7 and 14, and all 4 serum liver enzymes on d 7, 10 and 14. All parameters returned to baseline 2 weeks after cessation of dosing (d 28). MNTDC at 10 mg/kg/d did not have an effect on serum BSP retention or serum liver enzymes. At 25 mg/kg/d, MNTDC resulted in a slight increase in serum GGT levels on d 14. For androgens that exhibited liver toxicity, lower po doses were tested to determine a no observed effect level (NOEL). The NOEL for MT, DMAU, and MENT was 1 mg/kg/day. Comparison of %BSP retention across treatments indicated an overall ranking of the synthetic androgens from most to least hepatotoxic as: MT>DMAU>MENT>MNTDC. Hence, a promising model for early detection of potential liver toxicity by synthetic androgens has been developed. Funded by NIH NO1-HD-2-3338.

861 CLINICAL AND HISTOPATHOLOGIC EFFECTS OF ORAL FURAN EXPOSURE IN FISCHER-344 RATS.

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Furan is a chemical produced in foods during food processing and preservation techniques that involve heat treatment. Studies in both mice and rats have shown that furan is a potent hepatocarcinogen, causing a high incidence of hepatocellular- and cholangio-carcinomas. However, these studies did not define a threshold for tumor induction at the dose ranges tested. Hence, a subchronic oral toxicity study was conducted to identify a no-observed adverse effect level (NOAEL) in rats for hepatotoxicity and to better characterize non-neoplastic effects including those affecting hematology, clinical biochemistry, gross morphology and histopathology. Rats were exposed to furan by gavage for 5 days per week for 90 days using a lower furan dose range of 0.0, 0.03, 0.12, 0.5, 2.0 and 8.0 mg/kg bw/day. Liver, testes, spleen and kidney weights were significantly increased in the 8.0 mg/kg bw groups. Dose-dependent gross and histological changes in livers from different treatment groups correlated with changes in serum levels of the liver enzymes, ALT, ASP and ALK and with significant increases in the hepatocyte proliferation index as determined by immunohistochemical detection and quantification of Proliferating Cell Nuclear Antigen (PCNA). This is consistent with the histological changes in the liver. Although no alterations in thymus weights were observed with treatment, changes in cellularity were evident. The CD4+CD8+ cell population decreased, while CD4-CD8-, CD4+CD8- and CD4-CD8+ cells increased with dose, suggesting that thymic lymphocyte maturation was altered by treatment. This effect was more pronounced in male than female rats. The functional consequences of thymic changes are not known. In conclusion, our study shows that multiple organs can be affected by exposure to furan, suggesting that parameters other than hepatotoxicity may also need to considered when establishing the NOAEL for furan.

862 EFFECTS OF ORAL FURAN ADMINISTRATION ON LIVER GENE EXPRESSION AND PROTEIN EXPRESSION IN FISHER-344 RATS.

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Furan, formed by heat during food processing and canning, has been found in a wide variety of processed foods, including baby food, fruit juices and infant formula. Rodent toxicity studies and furan levels in food suggest furan as a potential human health hazard. The effects of furan on the expression of specific genes and proteins in liver were evaluated in a 90 day rodent study. Male and female Fischer-344 rats were gavaged 5 days a week for 90 days with furan dissolved in corn oil at 0, 0.03, 0.12, 0.5, 2.0 and 8.0 mg/kg bw. Animals were sacrificed and liver samples were processed for RNA, protein or immunohistochemistry. Analyses of hepatic gene expression in high dose (8.0mg/kg bw) male rats found >2-fold increased transcript levels of cyp1a1 and glutathione S-transferase-pi (GST-pi), but >2-fold lower transcript levels for cyp1a2, cyp2b1, cyp3a3, cyp3a11 and cyp2e1 compared to control animals. Female rats at the same dose had increased gene expression for cyp3a11, GST-pi, cyclin D1, p21 (waf1) and COX2 and decreased gene expression for cyp3a3 and cyp4a1. Gene expression at 2.0 mg/kg bw showed a >2-fold increase of COX2 in males and females. No changes were observed at lower doses of furan. Western blot analyses found no significant changes in hepatic cytochrome p450 protein expression, GST-alpha or GST mu. GST-pi protein expression increased significantly (>3-fold in male and female rats). The down regulation of cytochrome p450 genes suggests possible decoupling of the electron transfer pathway in liver. The increased expression of GST-pi and PCNA in male and female rat livers, and p21 and cyclin D1 in female rat livers are indicative of altered cell proliferation with potential for carcinogenesis at furan levels lower than previously reported. Induction of COX2 may imply an inflammation-mediated mechanism for furan carcinogenesis.

863 HIGH DIETARY INORGANIC PHOSPHATE AFFECTS LIVER DEVELOPMENT THROUGH ALTERING PROTEIN TRANSLATION, CELL CYCLE, AND ANGIOGENESIS IN DEVELOPING MICE.

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Inorganic phosphate (Pi) plays a key role in diverse physiological functions. Several studies indicate that Pi may regulate several pivotal genes expression including Akt through regulating Na/Pi co-transporter (NPT). Akt has emerged as a crucial regulator of widely divergent cellular processes including proliferation, differentiation, and angiogenesis. In current study, we elucidated the potential effects of high dietary Pi on liver of developing mice. Eight 2-weeks-old transgenic mice expressing

CMV-LucR-c-myc-IRES-LucF reporter gene were randomly separated into 2 dietary groups (4 mice/group); one group on a normal diet containing 0.5% Pi and another group on a high phosphate diet containing 1% Pi. These mice were fed on the specified diet for 4 weeks. Our results clearly demonstrate that high dietary Pi may affect the liver of developing mice through regulating Akt-related cap-dependent protein translation, cell cycle regulation, and angiogenesis. Our results support the hypothesis that Pi works as a critical signal molecule for normal liver growth and suggest that careful restriction of Pi consumption may be important in maintaining a normal development.

864 GLUTATHIONE DEPLETION FOLLOWING INTRAPERITONEAL INJECTION OF 2-CHLOROACRYLONITRILE.

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2-Chloroacrylonitrile (2-CAN) is a toxic chemical used as a raw material to manufacture pesticides. 2-CAN is volatile and there is a high potential for exposure if there is a spill or release. Although the toxic mechanism is not fully understood, one theory is that 2-CAN may be metabolized to cyanide. Current medical treatment for 2-CAN exposure is for assumed cyanide formation. A better understanding of the mechanism of 2-CAN toxicity will permit more appropriate treatments in the event of an accidental exposure. Glutathione is a major cellular antioxidant. With sufficient glutathione depletion, oxidative stress could lead to cell death. Male Sprague-Dawley rats were administered 2-CAN at 10 mg/kg or 40 mg/kg intraperitoneal (i.p.). At various time points following the injection, the animals were euthanized via cardiac puncture. Cardiac blood was collected for cyanide analysis. Liver and lung tissues were harvested and frozen with liquid nitrogen until analysis for glutathione was completed. Following i.p. administration of 2-CAN there was a glutathione depletion of greater than 30% in the lung and greater than 40% in the liver. Blood cyanide concentrations were less than 0.05 mg/ml. Hind-limb weakness/paralysis was also observed. Since cyanide was not generated in substantial concentrations following 2-CAN administration, the use of an antidote specific for cyanide for 2-CAN toxicity may not be effective. However, since 2-CAN induced glutathione depletion, a treatment targeting glutathione depletion or an inhibitor of 2-CAN metabolism could be effective.

865 CYTOTOXICITY AND CRM1 DOWN-REGULATION OF 4-(METHYLNITRO-SAMINO)-1-(3-PYRIDYL)-1-BUTANONE IN HUMAN BRONCHIAL EPITHELIAL CELL LINE.

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Tobacco-specific nitrosamine, 4-(methylnitro-samino)-1-(3-pyridyl)-1-butanone (NNK), is a known human lung carcinogen (Group 1). Nucleocytoplasmic shuttling factors (NSF), including Ran, Ran GTPase activating protein (RanGAP), Ran guanine nucleotide exchange factor (RanGEF)/regulator of chromosome condensation (RCC1), chromosomal region maintenance 1 (CRM1), and transportin1, have been suggested to play important roles in transporting tumor suppressor genes and/or oncogenes between the nucleus and cytoplasm, which are critical for regulating carcinogenesis. The objective of this study is to exam the cytotoxic effects and regulation of these NSF expression levels in human bronchial epithelial cell line BEAS-2B. Cell cytotoxicity was measured by 3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay and protein changes were analyzed by western blot. BEAS-2B cells were treated with 0.1, 1, 10, 100, 150, and 500 μM NNK for 4, 24, 48, 72 and 96 h. The inhibitory effects were dose and time dependent ($p<0.001$). The significant inhibitory effects were observed at 1 and 10 μM from 72 to 96 h and at 100, 150, and 500 μM from 24 h to 96 h ($p<0.05$). The highest inhibition rates were observed in cells treated with NNK at 96 h. Based on the cell cytotoxicity results, BEAS-2B cells were further treated with 10, 75 and 150 μM NNK for 24 and 72 h to evaluate the expression patterns of NSF by western blot analysis. No significant changes in protein expression levels were observed in Ran, RanGAP, RCC1, and transportin1 in cells treated with NNK as compared to controls. However, CRM1 expression was significantly lower in cells treated with 150 μM NNK at both 24 h (1.2 fold decrease, $p=0.003$) and 72 h (1.3 fold decrease, $p=0.019$) than those of controls, suggesting that CRM1 is the molecular target of NNK exposure. Since CRM1 is a nuclear export receptor for some cancer specific cargo-genes/proteins such as p53 and p27, down-regulation of CRM1 might play an important role in NNK-induced lung carcinogenesis.

866 DIETARY FAT MODULATION OF BENZO(A)PYRENE-INDUCED ADENOMAS IN COLON OF APC^{MIN}MICE.

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Every year 56,000 deaths are attributable to colorectal cancer in the US. The consumption of well-done red meat and saturated fats, rich in polycyclic aromatic hydrocarbons (PAHs), may be a causative factor. The objective of this study is to investigate whether the formation and progression of colon tumors in adult Apc^{Min}mice is influenced by the ingestion of saturated fat containing benzo(a)pyrene (BaP), a PAH compound. Treatment consisted of 50 and 100 μg/kg of BaP dissolved in peanut and coconut oil (representatives of unsaturated and saturated fats, respectively) administered to 6-week-old male Apc^{Min}mice daily via oral gavage for 60 days. At the end of exposure, mice were sacrificed; blood samples were collected; tissues were retrieved and preserved in 10% formalin for observation for gross pathological changes. A portion of these tissues were subjected to a liquid-liquid extraction method using water, methanol, and chloroform and analyzed by a reverse phase HPLC for BaP/metabolites. At both the doses used, the BaP metabolite concentrations in plasma, and tissues of mice that received BaP through saturated fat were greater when compared to their control and unsaturated counterparts ($P < 0.05$). The concentration of reactive metabolites such as BaP 7,8-diol, BaP 3,6-dione, and BaP 6,12-dione were high in blood, jejunum and colon of mice that ingested BaP through unsaturated fat relative to control and unsaturated fat counterparts ($P < 0.05$). An increased prevalence of adenomas in colon of mice that ingested BaP through saturated dietary fat compared to unsaturated fat and controls ($P < 0.05$) was noticed. Our studies therefore strongly suggest BaP's involvement in colon carcinogenesis and its potentiation by dietary fat (funded by the NIH grants 1S11ES014156-01A1, 5T32HL007735-12, 1RO3CA130112-01, and G12 RR03032).

867 CYP1C1 AND CYP1A EXPRESSION IN PAHs INDUCED *FUNDULUS* LIVER TUMORS.

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CYP1C is the newest member of the cytochrome P450 family 1, however the physiological significance of this gene, its inducers and metabolic functions are unknown. Our previous studies have focused on constitutive and benzo(a)pyrene (BaP)-induced CYP1C1 and CYP1A protein expression in *Fundulus* tissues. We have hypothesized that CYP1 genes may be involved in the molecular mechanisms of BaP carcinogenesis. *Fundulus* fry were exposed at 6-8 days postfertilization (dpf) and again at 13-15 dpf for 6 hr to control, 5 mg/L BaP, and 5 mg/L dimethylbenzanthracene (DMBA). Fish were euthanized at 24, 48, 72, 96 and 120 hr after exposure, and processed routinely for sectioning. Immunohistochemistry (IHC) will be performed with both CYP1 antibodies to demonstrate protein expression in fry and with the BPDE-DNA antibody for detecting BaP-DNA adduct formation. In order to further investigate the potential CYP1 expression in neoplastic tissue, exposed *Fundulus* were grown out for 8 months, necropsied, and examined for gross lesions. Livers were sectioned, and stained with hematoxylin and eosin for microscopic evaluation. Gross lesions were observed during dissection in 20% of the BaP-treated group ($n = 50$) and 35% of the DMBA-treated group ($n = 51$). Histopathologic findings included the presence of foci of cellular alteration (eosinophilic foci) and hepatocellular carcinoma. IHC results suggest induced CYP1A expression in both BaP- and DMBA-treated liver lesions, but CYP1C1 expression is still inconclusive. Our results suggest that teleost CYP1C, in addition to CYP1A should be further investigated for its role in carcinogen bioactivation. This project is supported by NIEHS funding: R01ES012710.

868 EFFECT OF METHYL SUBSTITUTION ON TOXIC EVENTS ELICITED BY BENZ[A]ANTHRACENE DERIVATIVES IN LIVER CELL LINES.

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Monomethylated benz[a]anthracenes (MeBaAs) are an important group of derivatives of polycyclic aromatic hydrocarbons.Little is known about the impact of methylation on the effects associated with activation of the aryl hydrocarbon receptor (AhR)-dependent gene expression and/or with tumor promotion. We studied

effects of MeBaAs on the above mentioned endpoints in rat liver cell lines and compared them with effects of both benz[a]anthracene (BaA) and the potent carcinogen 7,12-dimethylbenz[a]anthracene (DMBA). Methyl substitution enhanced the AhR-mediated activity of BaA derivatives determined in a reporter gene assay, as the relative potencies (REPs) of all MeBaAs were higher than that of BaA. REPs of 6-MeBaA and 9-MeBaA, two most potent MeBaAs, were more than two orders of magnitude higher than REP of BaA. All MeBaAs induced higher levels of cytochrome P450 1A1 mRNA, whereas both BaA and its derivatives had similar effects on expression of CYP 1B1 or aldo-keto reductase 1C9 in rat liver WB-F344 cells. In contrast to genotoxic DMBA, MeBaAs induced only low to negligible DNA adduct formation. Only 10-MeBaA induced apoptosis and accumulation of phosphorylated p53, which could be associated with induction of oxidative stress, similarly to DMBA. With exception of 10-MeBaA, all MeBaAs induced cell proliferation in contact-inhibited WB-F344 cells, which corresponded with their ability to activate AhR. 1-, 2-, 8-, 10-, 11- and 12-MeBaA were efficient inhibitors of gap junctional intercellular communication in WB-F344 cells. This mode of action, like a disruption of cell proliferation control, may contribute to tumor promotion. The present data showed that the methyl substitution significantly influences the effects of MeBaAs associated with AhR activation or tumor promotion. Supported by Czech Ministry of Education (2B06150)and Grant Agency of the CR (310/07/0961).

869 CARCINOGENICITY OF 4-AMINOBIPHENYL IN ARYLAMINE N-ACETYLTRANSFERASE NAT1/NAT2 NULL MICE.

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Aromatic amines such as 4-aminobiphenyl (ABP) are carcinogenic in humans and animal models. These chemicals, however, require metabolic activation to DNA-damaging electrophiles for their carcinogenicity to be observed. Among the pathways of aromatic amine biotransformation that may impact bioactivation, acetylation by the genetically variable arylamine N-acetyltransferases NAT1 and NAT2 has been linked to variation in aromatic amine cancer risk. Since NAT1 and NAT2 are capable of either detoxifying aromatic amines by N-acetylation or activating their hydroxylamine metabolites by O-acetylation, it is unclear how these enzymes participate in facilitating or preventing toxic responses to particular agents. A limitation of epidemiological cancer risk studies in humans is the challenge in accurately identifying and quantifying exposure to various aromatic amines. On the other hand, animal models may not accurately predict risk for humans due to species differences in substrate selectivities of the orthologous carcinogen detoxifying and bioactivating enzymes, and in the degree of functional impairment in allelic variant forms. To better define the role of acetylation reactions in modulation of aromatic amine toxicity, we created a strain of mice lacking both mouse Nat1 and Nat2, which are devoid of any detectable acetylating activity. These Nat1/Nat2 null mice are being tested in a neonatal mouse long-term carcinogenicity assay using ABP, which is known to cause liver tumors in this species. Preliminary data indicate that Nat1/Nat2 null mice show a 50% reduction in liver tumor incidence compared to wild-type mice following ABP exposure. A marked gender difference is also observed, with both wild-type and Nat1/Nat2 null female mice being much less susceptible to ABP-induced liver tumors than their male counterparts. Coupled with our previous observation that ABP in vivo clearance is unchanged in Nat1/Nat2 null mice, these results suggest that for ABP, the protection afforded to male Nat1/Nat2 null compared to wild-type mice may be due to deficient hydroxylamine O-acetylation.

870 BENZO[A]PYRENE-7, 8-QUINONE FORMS COVALENT-DNA ADDUCTS *IN VITRO* BUT NONE WERE DETECTED IN THE LUNGS OR LIVERS OF STRAIN A/J MICE *IN VIVO*.

S. Nesnow, W. Padgett, G. Nelson, W. Winnik, G. Lambert, M. H. George and J. A. Ross. *Environmental Carcinogenesis Division, U.S. EPA, Research Triangle Park, NC.* Sponsor: S. Hester.

Benzo[a]pyrene (B[a]P) is a potent human and rodent lung carcinogen. This activity has been ascribed in part to the formation of B[a]P-7,8-diol-9,10-epoxide (BPDE)-DNA adducts. Other carcinogenic mechanisms have been proposed: 1.] The induction of apurinic sites from radical cation processes, and 2.] The metabolic formation of B[a]P-7,8-quinone (BPQ) which can form covalent DNA adducts or reactive oxygen species. The studies presented here seek to examine the role of BPQ-DNA adducts in B[a]P–induced mouse lung tumorigenesis. BPQ-nucleoside and BPQ-nucleotide-3'-phosphate adduct standards of deoxyguanosine, deoxyadenosine, and deoxycytosine were synthesized. Using these standards we identified 8 BPQ-DNA adducts from the incubation of calf thymus DNA with BPQ by 32P-postlabeling analysis. For the in vivo studies, male strain A/J mice were injected intraperitoneally once with BPQ or B[a]P-7,8-diol at 30, 10, 3, or 0 mg/kg. Lungs and livers were harvested after 24 hr, the DNA extracted and subjected to 32P-postlabeling analysis. Additional groups of mice were dosed once with BPQ or B[a]P-7,8-diol at 30 mg/kg and tissues harvested 48 and 72 hr later or B[a]P (50 mg/kg, a tumorigenic dose) and tissues harvested 24 or 72 hr later. Current analyses indicate that no BPQ or any other DNA adducts were observed in lung or liver tissues 24, 48, or 72 hr after the BPQ 30 mg/kg treatment. B[a]P-7,8-diol gave BPDE-DNA adducts at all time points in both tissues and B[a]P treatment gave only BPDE-DNA adducts in the lung. In each case, no BPQ-DNA adducts were detected. Mouse body weights significantly decreased over time after BPQ treatment suggesting BPQ-induced systemic toxicity. BPQ does not form stable covalent DNA adducts in the lungs or livers of strain A/J mice, suggesting that stable BPQ-covalent adducts are not a part of the complex of mechanisms involved in B[a]P-induced mouse lung tumorigenesis. This abstract does not necessarily reflect EPA policy.

871 CURCUMIN LOWERS THE THRESHOLD OF P53 ACTIVATION AND SUBSEQUENT INDUCTION OF DNA DAMAGE RECOGNITION PROTEINS XPC AND DDB2.

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Long-term exposure to polycyclic aromatic hydrocarbons (PAHs) has been linked to lung cancer. The prototypical PAH derivative, benzo[*a*]pyrene diol epoxide (BPDE), is believed to play an important role in lung carcinogenesis by forming bulky adducts on DNA. Elimination of these lesions requires the nucleotide excision repair (NER) system, which is subdivided into two pathways: global genomic repair (GGR) and transcription coupled repair (TCR). While TCR specifically removes damage on transcribed strands of active genes, GGR repairs damage found throughout the entire genome. The stabilization and activation of the tumor suppressor protein p53 is a major response to DNA damage. It is proposed that activated p53 induces the expression of DNA repair genes of the GGR pathway. In particular, the DNA damage recognition proteins XPC and DDB2 are transcriptionally regulated by p53. Unfortunately low dose BPDE, which is relevant to physiological exposure, fails to activate p53 thereby exposing DNA to mutation induction. Here we show that adduct levels in lung epithelial cells with BPDE-induced DNA damage are related to p53 expression. Adduct levels in p53-expressing (p53+) cells treated with BPDE were much lower than in p53-deficient (p53-) cells. When p53-expressing (p53+) cells were pretreated with curcumin, a natural constituent in turmeric, a further reduction of BPDE-DNA adducts was observed only in p53+ cells. While expression of XPC and DDB2 increased in a dose dependent manner in p53+ cells, pretreatment with curcumin signaled their induction with lower BPDE doses. Collectively these results suggest that the enhanced removal of BPDE-induced DNA damage in curcumin pretreated cells may occur through enhanced induction of XPC and DDB2. Thus, curcumin may act as a chemopreventive agent by lowering the threshold of p53 activation and inducing XPC and DDB2 with lower doses of carcinogens. Supported by NIH grants 5R03CA119295-02 and 1P30ES014443-01A1.

872 FURAN-2-YL-3-PYRIDIN-2-YL-PROPENONE INHIBIT 7, 12-DIMETHYLBENZ[A]ANTHRACENE-INDUCED GENOTOXICITY IN MCF-7 CELLS.

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1-Furan-2-yl-3-pyridin-2-yl-propenone (FPP-3) is an anti-inflammatory agent with a propenone moiety and chemically synthesized recently. In this study, we examined that the chemopreventive effect of FPP-3 on 7,12-dimethylbenz[a]anthracene (DMBA)-induced genotoxicity in MCF-7 cells. FPP-3 reduced the formation of the DMBA-DNA adduct. DMBA induced CYP1A1 and CYP1B1 gene expression and enzyme activity were inhibited by FPP-3. It inhibited DMBA-induced aryl hydrocarbon receptor (AhR) transactivation and DMBA-inducible nuclear localization of the AhR. Induction of detoxifying phase II genes by chemopreventive agents represents a coordinated protective response against oxidative stress and neoplastic effects of carcinogens. Transcription factor NF-E2 related factor 2 (Nrf2) regulates antioxidant response element (ARE) of phase II detoxifying and antioxidant enzymes, such as glutathione S-transferase (GST) and NAD(P)H:quinone oxidoreductase (QR). FPP-3 increased the expression and enzymatic activity of GST and QR. Moreover, FPP-3 increased transcriptional activity of GST and QR. GST and QR induction and Nrf2 translocation by FPP-3 were blocked by the PKC inhibitor Gö6983, and the p38 inhibitor SB203580. These re-

sults reflected a partial role of PKCδ and p38 signaling in FPP-3-mediated GSTA and QR induction through nuclear translocation of Nrf2. Classically, chemopreventive agents either inhibit CYP metabolizing enzyme or induce phase II detoxifying enzymes. These results suggest that FPP-3 has a potent protective effect against DMBA-induced genotoxicity through by modulating phase I and II enzymes and that it has potential as a chemopreventive agent.

873 ORAL CANCER CHEMOPREVENTION BY LYOPHILIZED STRAWBERRIES IN HAMSTER CHEEK POUCHES.

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Oral cancers represent 2.5-3.0% of all cancers, worldwide. Prognosis for oral cancers is poor with 5- and 10-year survival rates of 59% and 44%. Even with advances in treatment, these survival rates have not changed dramatically in three decades. Since current long-term treatments are relatively ineffective, it is important to develop novel strategies for the prevention of oral cancer. Chemoprevention by natural food products, e.g., black raspberries, has been demonstrated to be a viable approach for aerodigestive cancer prevention.
The current study used the hamster cheek pouch model (HCP) to evaluate administration of lyophilized strawberries (LS) during the initiation and progression of oral cancer induced by 7,12-dimethylbenz(a)anthracene (DMBA). LS were incorporated into AIN-76A pellets at 5% and 10% and fed to hamsters before, during, and after carcinogen treatment (Complete Chemoprevention Assay; CC), or after establishment of premalignant lesions (Post-initiation Assay; PI). Animals were sacrificed at 12 weeks and examined for total lesions and tumor incidence and multiplicity.
LS at 5% and 10% inhibited tumor formation by 28% ($p<0.05$) and 41% ($p<0.01$) in the CC Assay, and 54% ($p<0.01$) and 36% ($p<0.01$) in the PI Assay, respectively. Total lesions (leukoplakias, papillomas, tumors) were inhibited in both assays. Histological analysis of HCPs demonstrated that 10% LS in the CC Assay inhibited moderate and severe dysplasias by 31% ($p<0.01$) and 34% ($p<0.01$), respectively.
These experiments provide strong evidence that LS prevent oral tumorigenesis under conditions mimicking those in former tobacco users. Furthermore, this study extends our data supporting the ability of natural food products to modulate oral carcinogenesis when delivered in a mode directly amenable to the human oral cavity. Translation of preclinical oral cancer chemoprevention data into patient intervention strategies is essential. Ongoing human clinical trials targeting oral cancer prevention and recurrence will further define the utility of LS chemoprevention.

874 INHIBITION OF MMP SECRETION, INVASION AND GROWTH OF TONGUE CANCER CELL LINE SC-255 BY A NUTRIENT MIXTURE.

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Oral cancer represents about 3% of all cancers. The American Cancer Society estimates that approximately 30,000 new cases of oral cancer are diagnosed annually in USA with about 20,000 cases occurring in men and 10,000 in women. Although the exact cause of tongue cancer remains unknown, it is associated with tobacco product use: cigarettes, cigars, pipes and smokeless tobacco. Treatment of tongue cancer is based on the stage of the disease and involves radiation, chemotherapy and surgery. Nutrient supplementation has been reported to support recovery from cancer and maintenance of good health. We investigated the effect of a novel nutrient mixture (NM) containing lysine, proline, ascorbic acid and green tea extract on human tongue cancer cell line SC-255 in vitro evaluating viability, MMP secretion, invasion and morphology. Tongue cancer cell line SC-255 (ATCC) was grown in modified Dulbecco Eagles medium with 10% fetal bovine serum and antibiotics and treated with NM at 0, 10, 50, 100, 500 and 1000 μg/ml in triplicate at each dose. Cells were also treated with PMA (200 ng/ml) for MMP-9 induction. Cell proliferation was assayed by MTT, MMPs by zymography, invasion through Matrigel, and morphology by H&E staining.
Zymography demonstrated two bands corresponding to MMP-2 and MMP-9. PMA treatment enhanced MMP-9 secretion. NM inhibited the secretion of both MMP-2 and MMP-9 in a dose dependent fashion, with total virtual inhibition at 500 μg/ml. Invasion through Matrigel was inhibited by 40%, 80% and 100% at 100, 500 and 1000 μg/ml NM respectively. NM was not toxic to SC-255 tongue cancer cell line at 100 μg, but exhibited 20 % and 30% toxicity at 500 and 1000 μg/ml. H&E staining showed slight changes at the highest concentration.NM significantly inhibited MMP secretion, invasion through Matrigel and growth, important parameters for cancer prevention, suggesting NM has potential for therapeutic use in treatment of tongue cancer.

875 THE EFFECT OF CIGARETTE SMOKE AND THE ANTIOXIDANTS RESVERATROL AND CURCUMIN ON A TWO-STAGE CELL TRANSFORMATION ASSAY.

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Reactive species detected within cigarette smoke (CS) can potentiate oxidative stress associated with the development of carcinogenesis. The Syrian Hamster Embryo (SHE) two-stage cell transformation assay provides an in vitro model for the earliest stage in carcinogenicity; morphological cell transformation (MT). Using this assay we have shown that CS can act at both the initiation and promotion stage of cell transformation. In this study, we have investigated the role of two antioxidants, resveratrol and curcumin, in the carcinogenic response to CS in the SHE transformation assay. METHOD: SHE cells were exposed to CS in the presence or absence of resveratrol and curcumin for either 24 hours (initiation phase) or 7 days (promotion phase). Benzo[a]pyrene (B[a]P) was used as a positive control initiator. Colonies were fixed, stained, and examined microscopically. The frequency of MT for each treatment group was compared to control CS treated group. RESULTS and CONLUSIONS: The results of this study demonstrate that CS increases MT of SHE cells at both the initiation and promotion stages. Addition of anti-oxidants curcumin and resveratrol reduced B[a]P and/or cigarette smoke induced MT in SHE cells up to 80% when compared to control smoke treated-cells. The two-stage SHE assay provides an alternative in vitro method for screening chemicals for CS induced MT.

876 CHEMOPREVENTION OF MURINE LUNG TUMOR PROGRESSION BY SULINDAC.

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We developed a transgenic mouse lung tumor model for the early stages of lung cancer. Treatment of mice with 500 ug/ml of doxycycline (DOX) results in inducible, lung specific expression of the human Ki-ras^{G12C} allele and the development of benign focal hyperplasias and adenomas in 9-12 months. After 9 months of DOX exposure, mice co-treated with 150 mg/kg (6 weekly ip injections) of the known lung tumor promoter, butylated hydroxytoluene (BHT), exhibited increased lung tumor multiplicity±SE (from 24.3±0.2 to 37.4±0.8) and a more progressive lung tumor phenotype, as seen by the shift from predominantly hyperplasia development in DOX only mice to larger, atypical adenomas in the DOX/BHT mice. The BHT mediated inflammatory response was associated with recruitment of iNOS and COX-2 expressing macrophages to the tumor, as assessed by immunohistochemistry (IHC). Lung lesions at 9 months exhibited oxidative damage, as elevated 2,4-dinitrophenylhydrazone, phosphorylated NF-κβ, COX-2, and iNOS staining was observed in the epithelial tumor cells. Semi-quantitative IHC data demonstrated statistically significant increases in proliferative signals (increased p-AKT, p-JNK, survivin, and Ki-67) as well as diminished apoptotic signals (p19, phosphorylated *p53*, and caspase-3 activity). When the anti-inflammatory agent sulindac was co-administered to DOX/BHT mice at 100 ppm in the diet for the entire study, sulindac abolished both the early recruitment of macrophages and the increase in iNOS, COX-2, and p-NF-κβ staining in the lung lesions, and also restored *p-p53* and p19 levels in the lung lesions as well. Sulindac also elicited increased caspase-3 activity along with inhibition of p-JNK, survivin, p-AKT, and Ki-67 levels but had little effect on tumor multiplicity. Thus, administration of sulindac to the at risk population of smokers who already have genetic damage as a result of chronic exposure to cigarette smoke may prevent the progression of these lesions to more severe phenotypes.
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877 3-METHYLTHIO-PROPIONIC ACID ETHYL ESTER ENHANCED DIFFERENTIATION IN WELL-DIFFERENTIATED HUMAN COLON CANCER CELLS.

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The consumption of fruit has been traditionally considered a risk-reducing factor in cancer causation, and has been attributed primarily to the high vitamin and fiber content, and to minor unidentified phytochemicals. The goal of this study was to identify phytochemicals from fruit that induce the differentiation of RCM-1 cells,

which is a well-differentiated human colon cancer cells. Confluent RCM-1 cells spontaneously form some villi-like duct structures in plastic culture dish. When RCM-1 cells, seeded in 96-multi-well plate, were cultured with phytochemical extracts, the number and size of duct formation were increased, concomitant with an increase in alkaline phosphatase, which is an early stage marker in cell differentiation. The phytochemical extract from the fully ripened fruit of Katsura-uri Japanese pickling melon (*Cucumis melo* var. *conomon*) was purified beginning with an *n*-hexane extraction, and followed by silica gel chromatography. The RCM-1 duct formation assay was used to assess the bioactive fractions. The most active fraction contained a compound identified as 3-methylthio-propionic acid ethyl ester, based on GC retention times, EI-MS, ^{1}H-NMR and ^{13}C-NMR spectra. Authentic 3-methylthio-propionic acid ethyl ester similarly enhanced duct formation in the tumorigenic RCM-1 cells, and enhance alkaline phosphatase activity by 1.3-2.5 times at concentrations of 0.25-2 mM, which were concentrations that did not show any cytotoxic effect. We concluded that 3-methylthio-propionic acid ethyl ester from *n*-hexane soluble fraction of Katsura-uri fruit has the ability to induce differentiation in the well-differentiated tumorigenic RCM-1 cell line, and could be an active anti-carcinogenic compound.

878 MODELING THE EFFECTS OF CHLORPYRIFOS ON *CAENORHABDITIS ELEGANS* GROWTH AND DEVELOPMENT.

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Currently, we are assessing the potential use of the nematode *C. elegans* as an alternative model organism in high-throughput chemical and genetic screening. In our assays, COPAS Biosort flow cytometry technology is used to measure the length or time of flight (TOF) and optical density or extinction (EXT) of individual nematodes under different chemical exposure conditions in 96 well plates. Chlorpyrifos is an organophosphate pesticide known to cause neurotoxicity due to cholinesterase inhibition in mammals as well as developmental delays. Because of its known developmental effects, chlorpyrifos was chosen as a model toxicant in growth assays using *C. elegans*. We exposed nematodes to chlorpyrifos (0.5-50 uM) and measured their development every 12 h from the first larval stage (L1s). Control nematodes matured from L1s to gravid adults by 72 h. A Markov model was used to describe the movement of distributions of log(EXT) and log(TOF) between 12h sampling times. Estimated growth rates were found to depend on the measured variables, with constant growth rates on subsets of the ranges. Although the growth rates within each subset were dose dependent, the subsets themselves were the same for all dose levels. Chlorpyrifos retarded development between nematode stages as well as growth within a given stage. Growth rates were highest at all concentrations up to 12 h, probably the result of an initial growth spurt as the starved hatchlings began to feed. Growth rates slowed somewhat for the progression through the remaining larval stages. The growth rate for adults was slower for the control nematodes and the lowest 2 doses, but increased for the higher doses. After 72 h at the higher doses, nematodes were young adults but were not yet reproductive and were therefore still growing at higher rates. The proposed model of the growth of *C. elegans* fits the data well and is able to distinguish different growth rates for earlier and later life stages.

879 TOXICITY OF DOXORUBICIN AND ISOPROTERENOL DEPENDS ON THE DIFFERENTIATION STAGE OF H9C2 MYOBLASTS.

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Rat H9c2 myoblasts are an in vitro model for both skeletal and cardiac muscle. However, H9c2 cells are able to differentiate from mono-nucleated myoblasts to myotubes when cultured in a low serum media, acquiring mostly a skeletal muscle phenotype. Addition of all-trans retinoic acid (RA) in low serum media allows the maintenance of the cardiac phenotype. Undifferentiated H9c2 myoblasts have been used to study the mechanisms of action of several cardiotoxicants, including the antineoplastic agent doxorubicin (DOX) and the beta-adrenoceptor agonist isoproterenol (ISO). In this context, the objective of the present study was to investigate whether the susceptibility of H9c2 myoblasts to ISO and DOX depends on the differentiation stage.
The differentiation of H9c2 myoblasts was promoted by reducing the serum concentration of the media to 1% in the presence or absence of RA. Undifferentiated control cells were maintained in 10% serum medium.
H9c2 cells cultured for 5 days in a low serum medium presented markers of differentiation, including expression of sarcomeric protein myosin heavy chain and decreased cell proliferation. We observed that differentiated cells are more susceptible to DOX treatment, with fewer cells surviving a 48 hours exposure. Interestingly, flow cytometer experiments revealed that differentiated cells incorporated a higher amount of DOX. In addition, the difference between the differentiation states was blurred when DOX concentration was increased. Similar increased susceptibility on differentiated H9c2 cells was observed for ISO, which causes an increase in apoptotic nuclei and a loss of differentiated cells for 24 and 48 hours. The addition of RA to low serum medium did not enhance DOX or ISO toxicity.
The present study suggests that when differentiated to skeletal and/or cardiac cells, H9c2 cells appear to be more susceptible to DOX and ISO than undifferentiated myoblasts.
Work funded by NIH HL58016 and FCT PTDC-QUI-64358-2006 (co-financed by the EC fund FEDER).

880 IDENTIFICATION OF HYPOXIA RESPONSE INDUCERS USING QUANTITATIVE HIGH-THROUGHPUT SCREENING.

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Tumor hypoxia is a hallmark of solid tumors. The hypoxic tumor microenvironment induces the Hypoxia-Inducible Factor (HIF)-1 signaling pathway, which allows continued tumor survival and growth by promoting glycolysis and angiogenesis. HIF-1 is a heterodimeric transcription factor composed of hypoxia-inducible HIF-1α and constitutively-expressed HIF-1β; HIF-1α protein expression is elevated in tumor cells that are the greatest distance from blood vessels. In hypoxic conditions, the two subunits dimerize, allowing translocation of the HIF-1 complex to the nucleus, where it binds to the Hypoxia-Response Element (HRE) and activates expression of target genes implicated in angiogenesis, cell growth and survival. In order to assess the effect of environmental chemicals on the HRE pathway, we screened a collection of 1408 known or suspected toxins from the National Toxicology Program (NTP) using a cell-based β-lactamase HRE reporter gene assay in a quantitative high throughput screening format. We identified ten hypoxia inducers, including known chemical hypoxic inducer, cobalt sulfate, and several other novel inducers. Active compounds were further confirmed in replicate assays and in a secondary assay of vascular endothelial growth factor (VEGF) secretion, which is transcriptionally regulated by HIF-1/HRE activity. These results demonstrate the effectiveness of qHTS at identifying novel hypoxia inducers, and suggest that the toxic effects of these compounds may be due in part to induction of hypoxia in certain cell populations or tissues.

881 ETHANOL TERATOGENESIS IN JAPANESE MEDAKA: EFFECTS AT THE CELLULAR LEVEL.

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The adverse effects of alcohol on the developing humans represent a spectrum of structural and neurobehavioral abnormalities, most appropriately termed as fetal alcohol spectrum disorder (FASD). The mechanism by which ethanol induces FASD is unknown. Human studies of FASD are very limited due to ethical constraints; however, several animal models from nematodes to mammals are utilized to understand the molecular mechanism of this disorder. We have used Japanese medaka (Oryzias latipes) embryo-larval development as a unique non-mammalian model to study the molecular mechanism of FASD. Fertilized medaka eggs were exposed to ethanol (0-400 mM) for 48 hour post fertilization (hpf) and then maintained in regular embryo rearing medium without ethanol. Viable embryos were harvested on 0, 2, 4 and 6 day post fertilization (dpf) and analyzed for DNA, RNA and protein contents of the embryos. By applying semi-quantitative RT-PCR (rRT-PCR) and quantitative real-time RT-PCR (qRT-PCR), RNA samples were further analyzed for seven transcription factors, emx2, en2, iro3, otx2, shh, wnt1 and zic5 which are expressed in the neural tube of medaka embryo during early phase of development. RNA and protein contents of the embryos were significantly reduced by ethanol at 400 mM dose on 4 and 6 dpf compared to the control (no ethanol), and 100 mM ethanol treated embryos. However, significant reduction of DNA was observed only in 4dpf embryos. Total protein contents of yolk remained unaltered after ethanol treatment. Expression pattern of emx2, en2, iro3, otx2, shh, wnt1, and zic5 mRNAs were found to be developmentally regulated, however, remained unaltered after ethanol treatment. It is therefore concluded that alteration of nucleic acid and protein contents of medaka embryo by ethanol could be used as an indicator of embryonic growth retardation which might be the result of disruption of specific gene function during development.

882 FROM YEAST TO HUMANS: FUNCTIONAL GENOMICS IN THE MODELING OF ARSENIC AND HEAVY METAL TOXICITY IN YEAST AND ITS RELEVANCE TO HUMANS.

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The parallel analysis of yeast mutants interrogates the growth phenotype of thousands of deletion strains simultaneously in a selective condition of interest. We have used this approach to establish the genetic requirements of yeast exposed to monomethylarsonous acid III (MMA^{III}), the metabolized form of arsenic that has been the focus of attention in recent years; and arsenite, an inorganic form present in drinking water. Exposure to these arsenicals identified different genes that significantly affected mutant growth at several concentrations and lengths of exposure, suggesting differences in their mechanisms of action and cellular responses against them. Glutathione was specifically required for protection against MMA^{III}, providing support of MMA^{III} inducing oxidative stress. In the case of arsenite, the requirement of several genes involved in DNA repair was indicative of DNA damage. We modeled the toxicity of these arsenicals by mapping the identified genes to the yeast interaction network and by Gene Ontology scoring. Based on the high degree of conservation shared between yeast and human genes, especially of the ones involved in basic metabolic processes, we extrapolated our findings to higher eukaryotes. In this context, we identified the SAS complex, functional homolog of the human *MYST1*, as highly essential for growth in arsenite. *MYST1* is a member of the MYST family of histone acetyltransferases that has an increasing number of connections to DNA repair and cancer. In addition, we have identified yeast genes essential for growth at toxic concentrations of cadmium, lead and zinc. Several of the identified genes have homologs in other species with similar, if not the same, biological function as their yeast counterparts, providing support that the mechanisms identified are likely to be relevant to mammals and, of particular interest, humans.

883 GENOTOXICITY TESTING USING THE MICRONUCLEUS ASSAY IN THE HUMAN EPIDERM™ 3D SKIN MODEL.

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Safety assessment of new products for human use requires genotoxicity testing to ensure their non-carcinogenicity. Current in vitro assays have low specificity resulting in a high rate of false positives that may be due to use of transformed cell lines, non-physiological exposures, and lack of normal metabolism. To help determine the biological relevance of chemicals that are positive in in vitro genotoxicity assays, in vivo assays are conducted. However, starting in 2009, European Union cosmetics manufacturers will not be able to use in vivo genotoxicity tests. To address this problem, more biologically relevant in vitro genotoxicity tests are needed. Since skin has the highest exposure to many cosmetic and personal care products, a skin model will better reflect human exposure and metabolism.

EpiDerm forms a 3D tissue that is highly reproducible, contains a skin-like barrier, and possesses biotransformation capabilities including CYP450, GST, and UDP enzymatic activity. The Micronucleus Assay (MNA) utilizing EpiDerm allows topical application of test materials in a similar fashion to actual human contact. Doses are given 24 hours apart in the presence of 3 μg/ml of Cytochalasin-B in the medium. Cells are harvested from the tissue 24 hours after the last dose. This protocol results in a reproducible population of binucleated cells (49.2% +/-9.5) with a low background % of micronucleated cells (0.08% +/-0.08). We have shown dose-related, statistically significant increases in micronuclei for 3 model genotoxins and for 6 additional rodent skin genotoxins. Also, 4 non-genotoxins were tested at concentrations resulting in <50% cell survival and shown to be negative. Finally, 3 genotoxins that require metabolic activation were positive in the MNA.

In conclusion, the MNA that utilizes EpiDerm with inherent metabolic activity is very useful for predicting genotoxicity in a structurally complex tissue of human origin.

884 GERM-LINE STEM CELLS-GONOCYTES AS AN *IN VITRO* MODEL FOR MALE DEVELOPMENTAL TOXICITY: COMPARISON FROM CYTOTOXICITY TO GENOMIC RESPONSES TO PHTHALATES.

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Gonocytes exist in the neonatal testis, represent a transient population of male germ-line stem cells. It has been shown that stem cell self-renewal and progeny production is probably controlled by the neighboring differentiated cells and extracellular matrix (ECM) in vivo known as niches. Recently, we developed an in vitro three dimensional gonocyte/Sertoli cells co-culture model with ECM, demonstrated this culture system creates an in vivo-like niche and supports germ-line stem cell functioning within a 3-D environment. In this study, we applied microarray-based gene expression to examine the effects of different phthalate esters (PE). Known in vivo male developmentally toxic PEs (DTPE) and developmentally non-toxic PEs (DNTPE) were evaluated. We observed that treatments with DTPEs induced significantly greater dose-dependent morphological changes, decrease in cell viability and increase in cytotoxicity than those treated with DNTPEs. In order to further compared these responses,gene expression changes were evaluated. We found a significantly greater number of genes changed in the DTPEs versus gene expression in cells treated with DNTPEs. Similar gene expression patterns (GEP) within the DNTPE or DTPE group were observed in a non-supervised cluster analysis. The GEP that resulted from our in vitro model were consistent with GEP in the rat in vivo study reported by Liu et. al (2005). Our systems-based GO-Quant analysis found significant alterations in cell cycle,phosphate transport, and apoptosis regulation with DTPE but not with DNTPE. In comparison with our cytotoxicity results, the global gene expressional analysis reveals a much more distinct pattern between DTPE and DNTPE response. In summary, our observations demonstrated that this germ-line stem cell-based model that captures both the 3-D organization and multicellular complexity of the target system may be useful in identifying responses of developmental reproductive toxicants.(EPA R826886,NIEHS 1PO1ES09601,R01-ES1063,P30 ES07033 and SOT CP Grants,CAAT and HSUS/P&G Award)

885 INTERLABORATORY VALIDATION OF PRECISION CUT LUNG SLICES AS PRE-STUDY TEST SYSTEM FOR INHALATION STUDIES.

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Introduction: Precision cut lung slices (PCLS) offer the opportunity to gain insight into lung physiology under in vitro cell culture conditions. PCLS facilitate to address cellular and functional responses in laboratory species and humans. Possible endpoints that can be assessed include cytotoxicity, release of chemokines and cytokines, and immunohistological characterization. Using this technique, inter-species comparisons and comparisons of large numbers of test substances can easily be performed thereby offering a way of reducing the number of experimental animals used. We aim to develop a strategy to employ PCLS as pre-study test system for in vivo inhalation toxicity studies. We therefore perform this inter-laboratory validation of the PCLS technique. The objective was to assess the pulmonary in vitro cytotoxcity and the release of inflammatory mediators induced by several test substances.

Methods: Rat PCLS were prepared essentially as described before, cultured under conventional cell culture conditions and exposed to LPS, Triton X-100. Lipopolysaccharide (LPS) was used as a maximal stimulus for inflammation and immune response and Triton X-100 was used as cytotoxic agent. After 24 hours of incubation, cytotoxicity parameters such as membrane integrity and mitochondrial activity (WST-1) were assessed, and the amounts of the inflammatory cytokine IL-1alpha were determined by ELISA.

Results: LPS triggered profound pro-inflammatory effects in PCLS as evidenced by changes of IL-1alpha. Treatment with Triton X-100 markedly increased the levels of LDH released and reduced mitochondrial activity.

Conclusion: PCLS show characteristic responses to typical pro-inflammatory stimuli and may thus provide a suitable in vitro technique to predict the immunomodulatory and cytotoxic potency of inhaled substances. In the second part of the validation study we will assess the toxicity of a set of ten test substances on PCLS.

886 GENE EXPRESSION PROFILING OF THE HUMAN MACROPHAGE TRANSCRIPTOME IN RESPONSE TO THE RESPIRATORY SENSITIZER HEXAMETHYLENE DIISOCYANATE.

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Occupational exposure to chemicals is one of the main causes of respiratory allergy and asthma. Identification of chemicals that trigger allergic asthma is difficult as underlying processes and specific markers have not yet been clearly defined. Moreover, adequate classification of the respiratory toxicity of chemicals is hampered due to the lack of a validated in vitro test method. The study of differential

gene expression profiles in appropriate in vitro cell systems are a promising approach to identify selective markers for respiratory allergy. The major goal of our research is to study selected human resident lung cell types in response to respiratory sensitizers and irritating compounds, and to identify genes that are able to discriminate between both classes of chemicals.
As alveolar macrophages have important immunological and inflammatory properties in response to foreign substances in the lung, in vitro cultures of human THP-1 cells which were differentiated into macrophages were studied. THP-1 macrophages were exposed to 55 µg/ml (IC20) of the respiratory sensitizer hexamethylene diisocanate during 6, 10 and 24 hours. Overall changes in gene expression were evaluated using oligonucleotide microarrays containing about 30000 human genes. Several genes which are involved in different biological processes were differentially expressed at the different time points of exposure as compared to unexposed macrophages. Further microarray experiments using human lung cell lines (A549 and Beas2B) after exposure to respiratory sensitizers may provide the necessary complementary information to allow identification of specific gene markers for respiratory sensitization.

887 GLOBAL GENE EXPRESSION ANALYSIS OF ORGANOPHOSPHATE INTOXICATION.

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The wide use of organophosphate (OP) pesticides makes them an important public health concern because of continuing issues in OP toxicity including off-target effects and the mechanism of neuronal degeneration. We exposed cultures of Caenorhabditis elegans to 3 neurotoxicants, dichlorvos, fenamiphos, and mefloquine, and assayed global gene expression using whole genome microarrays. Dichlorvos and fenamiphos are OPs that inhibit acetylcholinesterase reversibly and irreversibly, respectively, while mefloquine appears to perturb neuronal Ca++ homeostasis, providing an outgroup for analysis. Three replicate cultures were exposed to the toxicants in bacteria-free, liquid CeHR medium at doses that arrested the development of 10%, 50% or 90% of worms in the L4 stage at 24 h after exposure. After 8 h of experimental exposure, worms were harvested, mRNA was prepared, and global gene expression was assayed. Data reduction and RMA preprocessing were performed before statistical analysis. Only probe sets called "Present" in at least three samples using the MAS 5.0 algorithm and where at least one condition differed from control by 1.8 fold were retained. Mefloquine and the OPs display markedly different patterns of expression. As expected, the patterns of gene expression elicited by dichlorvos and fenamiphos are very similar. Eighty-eight genes were differentially expressed under OP but not mefloquine treatment. A large fraction of the genes affected by the OPs have functions in neuronal and muscle tissues, membrane lipid metabolism, or apoptosis/cell death. However, several cytochrome P450 genes respond differently to the two OPs. A distinct set of P450 and Phase I detoxification enzymes are induced by mefloquine. Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army. The research was sponsored by the U.S. Army Medical Research and Materiel Command, Military Operational Medicine Research Program.

888 A GENETIC APPROACH TO ARSENIC SUSCEPTIBILITY.

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Chronic exposure to arsenic-contaminated drinking water can lead to a variety of serious pathological outcomes. However, differential responsiveness within studied human populations suggests inter-individual genetic variation plays an important role. We are using Drosophila to study toxic metal response pathways because of unrivalled access to varied genetic approaches and significant demonstrable overlap with many aspects of mammalian physiology and disease phenotypes. Genetic analysis of wild type strains exhibiting relative sensitivity or resistance to the toxic effects of arsenic identified a limited X chromosomal region potentially responsible for conferring the differential phenotype. We created lines harboring small, overlapping deficiencies in this region to help find a specific gene (or genes) that could be responsible for tolerance/susceptibility. The analysis identified an arsenic-sensitive line in which the glutathione synthetase (GS) gene dose is half that of wild type flies. Knock-down of GS expression by RNAi in S2 cells enhanced sensitivity to arsenic, and a P-element mutagenized fly strain that produces reduced levels of glutathione (GSH) compared to wild type was extremely sensitive to arsenic. These analyses, initially derived from sampling natural variation, provide genetic proof that a functional GSH biosynthetic pathway is required in vivo for a robust defense against arsenic, and both complement and extend previous work on GSH-arsenic interaction in mammalian cells. We suggest that polymorphisms in GSH biosynthetic pathway genes could be a contributor to differential arsenic sensitivity in human populations. Metabolism of inorganic arsenic to methylated forms appears to be absent in Drosophila: to that end we have "humanized" Drosophila with the appropriate metabolic enzyme and are examining the consequences for chromosomal instability and other toxic endpoints. Overall, such approaches suggest that Drosophila can be used as a model to help shed light on the mechanisms by which cells respond to the toxic effects of arsenic at the organismal level.

889 EPIGENETIC CYTOTOXICITY OF METALS ON MOUSE EMBRYONIC STEM CELLS.

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This novel cell culture approach in *in vitro* toxicological screening employs the innate ability of pluripotent mouse embryonic stem (mES) cells (ES-D3, ATCC), derived from the cell mass of mouse blastocysts, to differentiate toward specific cell lineages. We examined the effects of metal exposure from 1- to 5-days (As, Mn, Li, Hg, Cd, Ni, Pb, Cu) on confluent cells derived from embryoid bodies (EBs) seeded on collagen type-I coated 96 well plates or collagen-coated 12-mm Millipore inserts. Effects were monitored using cell viability (MTT) and paracellular permeability (PP, [^{3}H]-mannitol, lucifer yellow; transmonolayer electrical resistance, TMER) assays, respectively. IC50s were calculated from concentration-effect curves and the data were subjected to Students' *t*-test for paired sample means. The data suggest that intact monolayers demonstrate high TMER (~700 Ω-cm^2) after 16-days in culture, indicating formation of tight junctions. In response to increasing concentrations of metals, cell viability of cells decreases, while PP of differentiated mES cells proportionally increases. Also, MTT and PP assays reveal that differentiated mES cells are more sensitive to equivalent concentrations of metals than previously studied Caco-2 cells in the same system. In addition, differentiated mES cells exposed to As and Cd exhibited a hormesis effect at lower concentrations of chemicals. The study also underlines the requirement of seeding density of less than 10,000 cells per well or insert on the ability of differentiated mES cells to form confluent intact monolayers with high TMER. Thus the mES cell culture model presents a valuable approach for utilization in *in vitro* cytotoxicity testing and drug development. (Supported in part by grants from ARDF, 35336-985; IFER, 35262-985; NIH AREA, R15-ES012170-01).

890 DEVELOPMENT OF NORMAL HUMAN COLON CELL CULTURES TO IDENTIFY UNREGULATED DISINFECTION BY-PRODUCTS (DBPS) WITH CARCINOGENIC POTENTIAL.

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Epidemiological studies have linked the consumption of chlorinated surface waters to an increased risk of colorectal cancer. Approximately 600 DBPs, less that half of the total organic carbon in drinking water have been identified. We are developing an in vitro system to identify DBPs with a potential to transform normal human colonocytes (NCM460 cells) into malignant cells. Halonitromethanes (HNMs) were selected and compared to DBPs demonstrated to induce colon cancer in laboratory animals. NCM460 cultures were characterized for cytotoxicity and genotoxicity induced by several classes of DBPs. The order of toxicities was HNMs>HAAs>THMs. Within a DBP series, the toxicity increased with the degree of bromination and decreased with the molecular weight. For cell transformation studies NCM460 cells were exposed to a non-cytotoxic concentration of azoxymethane (colon carcinogen), bromochloroacetic acid, tribromomethane, dibromonitromethane or tribromonitromethane for 3 – 10 days. Cells growing in suspension from all chemical treatments when subcultured formed colonies in soft agar (untreated cells did not), a characteristic of transformation. Microarray analyses were performed on NCM460 cells following treatment with the test chemicals. Early data showed that the Wnt signaling pathway and cell-cell adherens junctions were upregulated for all chemical treatments. These data suggest that while no toxicity data are available for the halonitromethanes, they may pose a carcinogenic risk since they share activation of pathways characteristic of the colon carcinogens. Proof of this would be obtained if the malignancy of the transformed cells is demonstrated in immunocompromised mice. This system would then prove useful in identifying priority, unregulated DBPs for carcinogenesis. (This abstract does not necessarily reflect EPA policy)

891 ICCVAM RECOMMENDATIONS ON THE USE OF FIVE *IN VITRO* PYROGEN TEST METHODS FOR ASSESSING THE POTENTIAL PYROGENICITY OF PHARMACEUTICALS AND OTHER PRODUCTS.

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Pyrogen testing is a critical step towards ensuring the safety of parenteral pharmaceuticals. The U.S., European, and Japanese Pharmacopoeias currently recognize two pyrogen tests, both of which require animals (i.e., the rabbit pyrogen test

[RPT] and the bacterial endotoxin test). Concerns for animal welfare have led researchers to develop alternative cell-based test methods that use human cells. ICCVAM recently evaluated the validation status of five in vitro test methods for assessing potential pyrogenicity of pharmaceuticals and other products as potential replacements for the RPT. These methods use IL-1 or IL-6 ELISAs to measure an increase in cytokines when human monocytoid cells (i.e., whole blood, isolated monocytes, or a Mono Mac 6 cell line) are exposed to Gram-negative endotoxin. The accuracy evaluation was based on 10 parenteral pharmaceuticals, each spiked with four concentrations of endotoxin. Accuracy ranged from 81% to 93%, false negative rates ranged from 1% to 27%, and false positive rates ranged from 3% to 23%. Quantitative and qualitative reliability analyses indicated that the test methods were generally reproducible within and among testing laboratories. Based on the results of these analyses, ICCVAM recommends that, while none of these five in vitro test methods should be considered as a complete replacement for the RPT, they could be considered to detect Gram-negative endotoxin in human parenteral drugs on a case-by-case basis, subject to product-specific validation by the appropriate regulatory agency. When used in this manner, these methods should further reduce the number of animals needed for pyrogenicity testing. ICCVAM recommends, consistent with U.S. Animal Welfare Regulations, that in vitro pyrogen tests must be considered prior to testing in animals and that an alternative test method be used when deemed appropriate.

892 UTILIZATION OF A RAT CARDIOMYOCYTE MODEL TO IDENTIFY HEART-SPECIFIC TOXICITY USING MULTIPLE ACUTE BIOCHEMICAL ENDPOINTS AND GENE EXPRESSION MARKERS.

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The present study was designed to evaluate a rat cardiomyocyte (CM) model using biochemical assays and gene expression markers to help identify heart-specific toxicity and differentiate risk between compounds. Understanding risk profiles for drugs with idiosyncratic toxicity was also important. Relative sensitivity of heart versus liver was assessed by using rat cardiomyocytes isolated from 1-2 day old neonates and a rat hepatoma (H4IIE) cell line. The antidiabetic drugs troglitazone (TRO), rosiglitazone (ROSI), and pioglitazone (PIO) were tested in both cell systems at exposure concentrations of 1, 5, 10, 20, 50, 100, and 300 μM for 24 or 72 hr. Cytotoxicity was determined using markers for acute toxicity, oxidative stress, and apoptosis. Gene expression markers were assessed in CM cells at 24 hr for regulation of apoptosis (BAX/Bcl2), glucose transports (GLUT1/GLUT4), and hypertrophy (atrial natriuretic peptide, ANP). In the H4IIE assays, all glitazones showed significant loss of total glutathione and inhibition of cell proliferation with a potency order of TRO>ROSI>PIO. In rat CM cells, cell death was observed at 300 μM TRO. No acute cytotoxicity was observed with ROSI or PIO, but there was a time-dependent decrease in total glutathione for all glitazones at 24 and 72 hr. An increase in the pro-apoptotic marker BAX was also observed in all glitazones with a concomitant suppression of the anti-apoptotic marker Bcl2. ROSI showed an increase in membrane lipid peroxidation at 24 and 72 hr. An increase in the hypertrophy marker ANP was also determined for ROSI at 24 hr that was not observed with TRO or PIO. In addition, ROSI showed a dose-dependent increase in GLUT1 and corresponding decrease in GLUT4. The GLUT1/GLUT4 reversal is characteristic of compounds known to cause cardiac hypertrophy. These results suggest that the rat CM model is a useful tool for identifying differences between compounds relating to cardiotoxicity and can be used to prioritize or rank-order compounds.

893 PREDICTING ACUTE ORAL TOXICITY (LD50) WITH AN *IN VITRO* CELL-BASED SYSTEM.

J. F. Pregenzer, K. L. Rutherford-Root, D. K. Petrella, S. B. Ouellette, A. J. Swanson, S. A. Thompson, P. C. Wilga and J. M. McKim. *CeeTox, Kalamazoo, MI.*

Recent initiatives, such as the Registration, Evaluation and Authorization of Chemicals (REACH), the Organization for Economic Co-operation and Development (OECD), and the 7th amendment to the European Cosmetics Directive, are focused on understanding chemical toxicity while at the same time reducing or replacing animal testing. Considerable progress has been made with regard to in vitro approaches for ocular and skin toxicity, however, there has been considerably less success using in vitro models to predict acute oral toxicity. The primary objective of this study was to evaluate the effectiveness of multi-endpoint analysis and dose-response to develop an algorithm that can provide an accurate estimate of the acute oral toxicity category. A rat hepatoma (H4IIE) cell line in combination with multiple endpoints for cell health in a 96-well format was used. The endpoints monitored were mitochondrial function, cell mass, cell death (membrane leakage), oxidative stress, apoptosis, CYP induction, and metabolic activation. Twenty five chemicals selected from published lists (OECD and ICCVAM) were evaluated in this system at 7 concentrations. The highest concentration was based on the limit of solubility in culture medium. Cells were exposed to compounds for 24 hours. The data obtained were processed with a new algorithm for processing the multiple parameter data sets and placed into toxicity categories based on definitions outlined by OECD guidelines. Concordance with LD50-based classification categories was greater than 80%. Compounds not accurately assessed by this model included those associated with neurotoxicity (aldicarb), or those that target specific receptors not present in the test system (nicotine). In conclusion, this screening approach yielded good estimates of acute oral toxicity and should be a useful tool for evaluating the oral toxicity of chemicals.

894 EVALUATION OF MORPHOLOGICAL CHARACTERISTICS AND NEURONAL FUNCTION USING HIGH CONTENT ANALYSIS.

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Neuronal cell morphology, including neurite outgrowth and count, cell body size, and formation of neural connections between cells, can be modulated by a variety of conditions such as trophic factors, electrical activity, synaptogenesis, and functional maturation and differentiation of neurons (Thoenen, 1991; Ooyen et al., 1995; Files and Nelson, 1992). Although *in vivo* models are widely used to study functionality, current utilization of *in vitro* neuronal assays have been known to increase overall treatment conditions with the ability to understand morphological function at the cellular level, while decreasing overall experimentation time from start to finish. Using fluorescent reagents and image analysis systems with neuronal-specific algorithms makes it possible to provide these benefits with the ability to automatically and objectively analyze the results for more robust assays.
The objective of this study was to characterize different primary neuronal cells (cortical, striatal, and hippocampal) by evaluating overall cell body and neurite morphological differences (i.e., cell body shape, neurite length, branching pattern, etc.), as well as assessing functional characteristics of synaptic markers. Differences in morphology using compounds known to inhibit neurite outgrowth were also evaluated. This treatment illustrated decreases in overall neurite growth, number of branches, and general morphological characteristics as compared to the untreated control wells. Using fluorescent staining and automated image analysis, we were able to accurately characterize neuronal cultures with respect to their morphological characteristics as well as neuronal function, thus making it easier to conduct in-depth classification of primary neuronal cultures.

895 DRUG METABOLIZING ENZYME ACTIVITY IN HUMAN *IN VITRO* DERMAL (EPIDERM) AND AIRWAY (EPIAIRWAY) EPITHELIAL MODELS.

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Human dermal and airway epithelia contain xenobiotic metabolizing enzymes (XME) including a variety of phase I (oxidative) and phase II (conjugative) activities. These XMEs play an important role in biotransformation of drugs and environmental/occupational chemicals. Biotransformation of chemicals may lead to altered drug activity or formation of toxic/mutagenic metabolites. The present work evaluated expression of XMEs in highly differentiated in vitro models of human dermal (EpiDerm) and airway(EpiAirway) epithelia that are cultured at the air-liquid interface to facilitate in vivo-like chemical exposures. RT-PCR experiments were conducted to evaluate baseline and inducible expression of CYP isoforms in the epithelial cultures. EpiAirway cultures constitutively expressed CYP1A1 (weak), CYP1B1, CYP2A6, CYP2B6 (weak), CYP2C8 (weak), CYP2C19, CYP2D6, CYP2E1 and CYP3A5, while CYP3A4 and 3A7 were not detected. 3-Methylcholanthrene (3MC) strongly increased expression of CYP1A1 and slightly increased CYP2B6 and CYP2C8 expression in EpiAirway. In EpiDerm, CYP1B1, CYP2C19, CYP2D6, CYP3A4 (weak) and CYP3A5 were constitutively expressed. 3-Methylcholanthrene (3MC) strongly increased expression of CYP1A1 and CYP 1B1 in EpiDerm. Enhanced metabolism of the CYP1A1 and CYP1B1 substrate ethoxyresorufin confirmed increased activity following treatment with 3MC. Thus CYP expression in EpiAirway and EpiDerm showed a high concordance with CYP expression reported for in vivo human airway and dermal epithelia. Total GST activity in the epithelial models was also evaluated by measuring conjugation of glutathione with 1-chloro-2,4-dinitrobenzene and UDP-Glucuronyltransferase activity was determined by 4-methylumbellipherone conjugation. High baseline GST and UDP-glucuronyltransferase activity in both models was not further enhanced by 3MC treatment. The results demonstrate that the EpiDerm and EpiAirway in vitro human epithelial models possess in vivo-like XME activities and may thus be useful for evaluating epithelial metabolism of drugs and environmental/occupational chemicals.

896 WHEN ACCOUNTING FOR BIOAVAILABILITY IN VITRO MATTERS.

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As the toxicological risk assessment paradigm is shifting from a black-box animal based approach to a more mechanistic approach using animal alternatives including *in vitro* assays, it is important to understand the factors that determine the variation in *in vitro* and *in vivo* toxicity data. Difficulties arise when extrapolating *in vitro* derived toxicity data (e.g. EC50s in cytotoxicity tests) to *in vivo* toxicity data (e.g. LD50s) because of the high variability of the *in vitro* data. Moreover, in a number of cases *in vitro* systems have shown to be less sensitive. Bioavailability differences of the test compound between *in vitro* and *in vivo* systems and within *in vitro* systems may help explain part of this variability and sensitivity difference. Thus, the aim of this study was to determine which *in vitro* system components and physico-chemical properties influence the free (bioavailable) concentration of a test compound in an *in vitro* system. Solid phase microextraction (SPME) was used to measure the partition coefficients of a range of polycyclic aromatic hydrocarbons to different *in vitro* assay components (e.g. serum, well plate plastic, headspace and cell lipids). The relative influence each *in vitro* system component has on the free concentration of the compound was assessed, modeled on the basis of the physico-chemical properties of the test compound, and linked to effect concentrations (EC50) in a range of assays including the chemically activated fluorescence expression (CAFLUX) and Neutral Red Uptake (NRU) assays. Results indicate that the free concentration of volatile (approximated by Henry's Law Constants, HLC) and hydrophobic compounds (approximated by Log P) are significantly reduced in a typical *in vitro* set-up as they readily evaporate and bind to *in vitro* matrices such as serum protein. Indeed, the free concentration, which may be modeled by Log P and HLC, is a better measure of exposure in *in vitro* assays as it reduces the influence system components have on the measured effect.

897 THE TEMPORAL AND DOSE GENOMIC RESPONSE OF A HUMAN PROSTATE CARCINOMA CELL LINE TO 5 ALPHA-DIHYDROTESTOSTERONE.

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The establishment of in vitro systems to determine the potential androgenic activity of chemicals of interest is indispensable in order to develop reliable high-throughput screening assays and to minimize animal testing studies for this mode of action. With this goal in mind, we have determined the genomic response of the 22Rv1 cells to a potent androgen receptor (AR) agonist, 5α-dihydrotestosterone (DHT) exposure in a time and dose dependent manner. DHT is generated by a 5-α reduction of testosterone, and unlike testosterone, DHT cannot be aromatized to estradiol therefore DHT is considered a pure AR agonist. The DHT concentrations tested were: 1fM, 10pM, 10nM, and 1μM. The time points were 8, 24 and 48h. The expression profiles were compared between treatment groups and controls (vehicle–treated) using Affymetrix Human Genome U133plus2.0 arrays. The analysis of the data indicates that the genomic response of the 22Rv1 cells to a potent AR agonist exposure is time and dose dependent. Similar characteristics of the genomic response are elicited by testosterone in an in vivo model (the fetal rat testis). By trend analysis, we determined that more than 2500 genes changed their expression by exposure to DHT ($p \leq 0.0001$ and $fc \leq -1.5$ or $fc \geq 1.5$). Using the annotation available, we determined that the response of the 22Rv1 cells to DHT involves changes in the expression of genes whose products are implicated in multiple cellular processes including cell proliferation, differentiation and tissue remodeling. Some of these genes have been previously identified to be androgen responsive, such as ACPP (PSA), PSMA, PART1, IGF1, STEAP4, POV1 (SLC43A1) and PSCA an emerging important diagnostic marker and therapeutic target in prostate cancer, among others. These data indicate that the 22Rv1 cell line is capable of mounting a proper androgenic response and they seem to offer a robust in vitro system to evaluate the potential androgenicity of chemicals of interest.

898 *IN VITRO* CYTOTOXICITY TEST METHODS FOR ESTIMATING RAT ACUTE ORAL TOXICITY: PREDICTION OF GHS ACUTE ORAL HAZARD CATEGORIES.

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An international validation study assessed the use of IC50 values from two in vitro neutral red uptake (NRU) basal cytotoxicity test methods (3T3 murine fibroblasts or normal human keratinocytes) for predicting acute oral hazard categories. Accuracy of these predictions (based on the United Nations Globally Harmonized System [GHS] hazard classifications) was evaluated using these IC50 values in two IC50-LD50 regressions developed from the 282 rat oral LD50 values and corresponding IC50 values from the Registry of Cytotoxicity (database of 347 chemicals with rodent data). Regressions were based on molar units and weight units of chemicals. Predicted LD50 values, calculated using NRU IC50 values in the regressions, were used to classify 67-68 chemicals into GHS categories. Accuracy is the proportion of correct outcomes (i.e., GHS category based on the in vivo rat oral LD50 value) for the in vitro predictions of GHS category. Accuracy of both methods and regressions for predicting GHS categories ranged from 29% (20/68) - 31% (21/67). Toxicity was overpredicted for 33% (22/67) - 40% (27/68) and underpredicted for 31% (21/68) - 36% (24/67) of the chemicals. Accuracy was highest for chemicals in the 300< LD50 ≤2000 mg/kg range (75% [12/16] - 81% [13/16]). Toxicity for this range was overpredicted for 19% (3/16) - 25% (4/16) and underpredicted for 0% (0/16) - 6% (1/16) of these chemicals. Accuracy was lowest for chemicals with LD50 ≤5 mg/kg (0% [0/6]). This study indicates that in vitro basal cytotoxicity tests are not sufficiently accurate when used alone for predicting GHS classification. However, all in vitro predictions for the nontoxic GHS category, LD50 >5000 mg/kg, matched the in vivo category, suggesting that acute oral toxicity testing for such chemicals could proceed directly to the limit test and thus reduce the number of animals tested. Supported by: NIEHS contracts N01-ES-35504, N01-ES-75408; EPA IAG DW-75-93893601-0; European Commission 19416-2002-04 F2ED ISP GB.

899 AN ALTERNATIVE USE OF *IN VITRO* CYTOTOXICITY TEST DATA TO DETERMINE WHEN RAT ACUTE ORAL TOXICITY TESTING SHOULD START WITH THE LIMIT TEST.

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Depending on the specific regulatory needs, acute oral toxicity test guidelines (i.e., the Up-and-Down Procedure [OECD TG 425] and the Acute Toxic Class method [OECD TG 423]) indicate that substances suspected to have LD50 > 2000 mg/kg or LD50 > 5000 mg/kg should be tested with the corresponding limit test using a small number of animals to confirm their lack of toxicity. Graphical plots of in vitro basal cytotoxicity IC50 (μg/mL) values vs. LD50 (mg/kg) values for the 418 Registry of Cytotoxicity chemicals with rat oral LD50 data were used to determine if the IC50 values could predict whether chemicals should be tested with the limit test instead of the main test, thereby reducing the use of animals. Chemicals with LD50 values less than the limit doses were categorized as positives (toxic) while those with LD50 values greater than the limit doses were categorized as negatives (nontoxic). Counts of chemicals above and below targeted IC50 cutoff values were used to calculate performance characteristics for the prediction of positive/negative. IC50 values ranging from 1 to 10,000 μg/mL were used to predict positive/negative at LD50 > 2000 mg/kg or LD50 > 5000 mg/kg. In each case, high false negative rates would be associated with an increase in the number of animals that die during testing, while high false positive rates would be associated with an increase in the total number of animals tested. For both limit doses, high IC50 values (≥ 1000 μg/mL) yielded high (≥ 79%) accuracy and sensitivity (i.e., low false negatives), but with low (< 63%) specificity (i.e., high false positives). Conversely, low IC50 values (≤ 60 μg/mL) yielded high (≥ 85%) specificity (i.e., low false positives), but low (≤ 62%) accuracy and sensitivity (i.e., high false negatives). These data can be used to identify the optimal IC50 values to indicate that testing should start with the limit test. ILS staff supported by NIEHS contract N01-ES-75408.

900 COMPARISON OF HUMAN AND RODENT 3-D LIVER TISSUE CO-CULTURES IN ADMET ASSESSMENT OF NEW DRUG CANDIDATES.

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Human and Rodent 3-D Liver Tissue Co-cultures expressing sustained tissue-specific function were developed to replace the short-term poor predictive function of industry-standard hepatocytes in metabolism and toxicity (ADMET) assessment of new drug candidates. Human, rat and mouse liver tissue co-cultures were grown in multiwell plates by seeding all of the cells of the native liver on a three-dimensional interconnecting porous scaffold, enabling cell-cell interaction and cell conformation to induce expression of extracellular matrix proteins and cytokines, recreating liver tissue structure and function in vitro. The liver tissues were exposed to model drugs and chemical toxins and their metabolic, genomic, toxicologic and functional response compared to hepatocytes grown from the same donor tissue. Hepatocyte cultures included suspension and monolayer growth on collagen or Matrigel®. Co-cultures from all three species formed native-like liver tissue structures and maintained liver-specific function for months as assessed by cell viability, protein expres-

sion (albumin, transferrin, fibronectin) and response to toxins. Cytochrome P450 (CYP450) induction and inhibition as well as drug metabolism function were also maintained for months, enabling chronic dosing concentrations, serial dosing and drug-drug interaction studies. Co-cultures were dosed at various culture ages as well as repeat-dosed weekly with the same inducer/inhibitor combinations over as much as three months to verify maintenance of CYPP450 function and to test hysteresis effects of repeat dosing. Hepatocyte cultures grown from the same donor were shortlived, expressed less tissue function and rendered higher assay variability, as expected from a decaying culture system. The liver tissue surrogate system replaces hepatocytes to provide an off-the-shelf, reproducible, specific system for ADMET assessment that enables chronic toxicity assessment as well as previously unattainable long-term human prediction including bioavailability and drug-drug interaction studies.

901 NON INVASIVE HIGH RESOLUTION *IN VIVO* IMAGING OF ALPHA-NAPTHYLISOTHIOCYANATE (ANIT) INDUCED HEPATOBILIARY TOXICITY IN STII MEDAKA.

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A novel transparent stock of medaka (Oryzias latipes; STII), recessive for all four pigments (iridophores, xanthophores, leucophores, melanophores), permits transcutaneous, high resolution (< 1μm) imaging of internal organs and tissues in living individuals. We applied this model to in vivo investigation of α-napthylisothiocyanate (ANIT) induced hepatobiliary toxicity. Distinct phenotypic responses to ANIT involving all aspects of intrahepatic biliary passageways (IHBPs), particularly bile preductular epithelial cells (BPDECs), associated with transitional passageways between canaliculi and bile ductules, were observed. Alterations included: attenuation/dilation of bile canaliculi, bile preductular lesions, hydropic vacuolation of hepatocytes and BPDECs, mild BPDEC hypertrophy, and biliary epithelial cell (BEC) hyperplasia. Ex vivo histological, immunohistochemical, and ultrastructural studies were employed to aid in interpretation of, and verify, in vivo findings. 3D reconstructions from in vivo investigations provided quantitative morphometric and volumetric evaluation of ANIT exposed and untreated livers. The findings presented show for the first time in vivo evaluation of toxicity in the STII medaka hepatobiliary system, and, in conjunction with prior in vivo work characterizing normalcy, advance our comparative understanding of this lower vertebrate hepatobiliary system and its response to toxic insult. While our focus has been the hepatobiliary system, other organ systems are equally amenable to in vivo study, and we consider the potential for discovery, within the context of in vivo investigation in STII medaka, as significant.

902 EFFECT OF SORAFENIB, SUNITINIB, IMATINIB AND DASATINIB ON MITOCHONDRIAL FUNCTION IN ISOLATED RAT LIVER AND HEART MITOCHONDRIA AND IN HEPG2 AND H9C2 CELLS.

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Cardiovascular toxicity has recently been suggested as a potential complication of cancer treatment with kinase inhibitors. The mechanisms leading to the cardiotoxicity are postulated to be mitochondrial dysfunction, either as a primary or secondary effect. We have developed several assays to investigate drug effects on mitochondrial function. Here we examine the effects of Sorafenib, Sunitinib, Imatinib and Dasatinib on respiration of isolated liver and heart mitochondria, inhibition of immunocaptured OXPHOS complexes, and viability of HepG2 and H9C2 cells. Of the four kinase inhibitors, only Sorafenib profoundly impaired mitochondrial respiration at concentrations below its therapeutic dose, whereas Sunitinib, Imatinib and Dasatinib had minimal effects on respiration only at concentrations greatly exceeding therapeutic levels. Assessment of OXPHOS complex activities indicated that Sorafenib potently inhibited C-I, IV & V, while Sunitinib, Imatinib and Dasatinib had effects at concentrations exceeding the therapeutic range. In accord with these findings, Sorafenib most potently killed both HepG2 and H9C2 cells grown in galactose as compared to cells grown in glucose, conditions that reveal mitochondrial dysfunction liabilities, whereas the others were equally toxic in both conditions. These data indicate that Sorafenib directly impairs mitochondrial function at therapeutic concentrations. Such mitotoxicity contributes to the drug's cytototoxicity, but in some patients, especially in combination with doxorubicin, could also contribute to idiosyncratic organ toxicity.

903 BIGUANIDE-INDUCED MITOCHONDRIAL DYSFUNCTION INCREASES LACTATE PRODUCTION AND REDUCES VIABILITY OF AEROBICALLY POISED HEPG2 AND HUMAN HEPATOCYTES IN CULTURE.

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Biguanides are used to lower blood glucose in both type I and 2 diabetes, although mode of action remains elusive. As a class, they induce lactic acidosis, a hallmark of mitochondrial impairment, with rank order of potency of phenformin > buformin >> metformin. To assess potential mitochondrial liabilities of these drugs, we evaluated their effects on: 1) mitochondrial respiration, 2) metabolic poise of HepG2 and primary human hepatocytes, and 3) viability of HepG2 cells grown in galactose to reveal mitochondrial effects, and 4) activities of the respiratory complexes. Respiration by isolated rat liver mitochondria is potently inhibited by phenformin, and less so by buformin. Preincubation (30min) exacerbated these effects, and was required to reveal inhibition by metformin. Metabolic profiling corroborated respiration inhibition, but also revealed compensatory increases in lactate production from accelerated glycolysis. HepG2 and human hepatocyte viability also reflected the above potency rank. Importantly, HepG2 cells grown in galactose are markedly more susceptible to biguanide toxicity compared to cells grown in glucose, indicating mitochondrial toxicity as a primary mode of action. Finally, mM concentrations of drugs were needed to inhibit immunocaptured respiratory complexes. This supports the notion that bioaccumulation of these molecules is required for toxicity, as reflected by excacerbation of respiration inhibition following preincubation. These data indicate that biguanide-induced lactic acidosis can be attributed to acceleration of glycolysis in response to mitochondrial impairment. Indeed, the desired clinical outcome – decreased blood glucose - could also be due to increased glucose uptake and utilization via glycolysis in response to drug-induced mitochondrial dysfunction.

904 ANALYSIS OF MITOCHONDRIAL FUNCTION BY MONITORING INTRACELLULAR OXYGEN CONSUMPTION.

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Knowledge of fluctuations in intracellular oxygen concentrations can shed particular light on mitochondrial function, as well as on the complex interrelationships in which the mitochondrion is involved. Such knowledge can be particularly valuable when attempting to assign mechanisms of toxicity. However, this type of analysis has traditionally been restricted, due in large part to the invasive and consumptive nature of conventional intracellular potentiometric measurement approaches. Here we present an intracellular oxygen-sensing methodology that addresses these limitations. It is based on photo-stable long-decay phosphorescent oxygen-sensitive probes, loaded passively into mammalian cells using fusion peptides and subsequently analysed using standard live cell imaging platforms. Probe emission is dynamically quenched by dissolved oxygen, whereby decreasing dissolved oxygen concentrations results in a reversible increase in probe signal. The approach facilitates the analysis of transient changes in intracellularly oxygen concentrations thereby allowing increased levels of detail on cell and mitochondrial function to be obtained. The effect of treatment with classical modulators of intracellular oxygen is presented as well as treatment with more physiologically relevant compounds including Ca2+ channel agonists (ryanodine and 4-chloro-m-cresol), inhibitors of specific components of the electron transport chain and different NO donors. Furthermore the potential for using fluorescence lifetime imaging microscopy (FLIM) for quantitative assessment of intracellular oxygen is explored.

905 A NOVEL METHOD FOR IDENTIFYING DRUG-INDUCED MTDNA DEPLETION AND MTDNA-ENCODED PROTEIN DEPLETION IN MITOCHONDRIA.

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Drug-induced mitochondrial toxicity can occur as a result of mtDNA depletion (nucleoside reverse transcriptase inhibitors, NRTIs) or direct inhibition of mtDNA-encoded protein synthesis (linezolid, chloramphenicol). Inhibition of mtDNA-encoded protein synthesis has, until now, been analyzed by in vitro assays which utilize the incorporation of [35S]-methionine, [3H]-leucine or other radioactive amino acids in isolated mitochondria. Lateral flow dipstick immunoassays

which measure the level of a mtDNA-encoded protein as well as a nuclear DNA-encoded protein enable simple and rapid determination of the ratio of these 2 proteins and, hence, identify impaired mtDNA-encoded protein synthesis. We have analyzed mitochondrial toxicity caused by antibiotics and NRTIs in cells, using dipsticks which compare the levels of Complex IV (cytochrome c oxidase), a mitochondrial protein which has 3 subunits encoded by mitochondrial DNA (mtDNA) and made by mitochondrial ribosomes, with that of frataxin, a mitochondrial protein encoded by nuclear DNA and made by cytosolic ribosomes. The ratio of Complex IV: frataxin decreases when a drug inhibits mtDNA replication or mtDNA-encoded protein synthesis. We confirmed the results obtained by the lateral flow dipstick immunoassays by Western blotting and immunocytochemical analysis. Measurements via these dipsticks require very small amounts of material (~ 2 microgram cell extract), are simple, rapid and quantifiable. It is envisaged that these dipstick assays will enable robust assessments of mitochondrial dysfunction both during drug development and in the clinic.

906 MITOCHONDRIAL LIABILITIES- A POSSIBLE EXPLANATION FOR IDIOSYNCRATIC HEPATOTOXICITY OBSERVED WITH NEFAZODONE, BUT NOT WITH BUSPIRONE AND TRAZODONE.

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Nefazodone was withdrawn from the US market in 2002 due to hepatic injury. The idiosyncratic nature suggests that genetic components could possibly play a role in the onset of hepatic injury. Mitochondrial liability has been shown for a variety of drugs (Dykens and Will, 2006/2007). It has been shown that toxicity for antibiotics could be linked to mitochondrial DNA mutations (Fischel-Ghodsian 2005). Here we show that Nefazodone impairs mitochondrial respiration in isolated rat liver mitochondria and identified possible mitochondrial targets. Nefazodone potently inhibited complex I, whereas buspirone and trazodone had no adverse effects. In addition, we found that Nefazodone was much more toxic to galactose-grown cells than to glucose grown cells. In contrast, buspirone and trazodone had equal toxicity in both cell lines and their toxicity was significantly less than what we observed with Nefazodone. Metabolic profiling, using Seahorse technology, further confirmed the cell data as we detected a decrease in oxygen consumption with a parallel increase in acidification for Nefazodone, but not for buspirone or trazodone. To investigate the influence of mtDNA mutations, the effect of Nefazodone on cell viability of galactose-grown fibroblast cell lines from 2 patients harboring a complex I ND1 mutation and ATPase6 mutation, respectively, were tested . Both cell lines were less susceptible to Nefazodone in comparison with the control cells suggesting that both mutant cell lines were less susceptible to impairment of oxidative phosphorylation and were able to rely more on glycolysis in comparison with the control cells. In summary, our data suggest that mitochondrial toxicity contributes significantly to the idiosyncratic etiology of Nefazodone toxicity.

907 EFFECT OF MAINSTREAM CIGARETTE SMOKE GAS/VAPOR PHASE AND WET TOTAL PARTICULATE MATTER ON THE METABOLIC PATHWAYS OF HUMAN LUNG EPITHELIAL CELLS: THE 'METABOLOMIC' APPROACH.

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Metabolomics is an emerging technology for identification and quantification of biochemicals across numerous metabolic pathways. One use is to identify on- and off-target disease-specific biomarkers following drug exposure in clinical studies. Herein, we identified changes in biochemical profiles of human alveolar epithelial carcinoma A549 cells following *in vitro* exposure to the complex mixture of compounds comprising cigarette smoke. A549 cells were exposed to wet total particulate matter (WTPM) or gas/vapor phase (GVP) of mainstream whole smoke from 2R4F reference cigarettes for 1 or 24 h at 0, 5, 25 or 50 µg/mL final concentration (or GVP equivalents) in serum-free Ham's F-12 medium. Cell pellets and selected media samples were then analyzed for perturbations in biochemical profiles, with named biochemicals measured, analyzed and reported in a "heat-map" format, along with biochemical and physiological interpretations (*m*Select, Metabolon Inc.). Both WTPM and GVP exposures appeared to decrease glycolytic flux, and increase oxidative stress, cell damage and possibly apoptosis. Interestingly, GVP appeared to predominantly induce early effects (1 h) while WTPM induced late effects (24 h). Alterations in Krebs cycle, urea cycle and energy and coenzyme A metabolism were unique to WTPM exposure, while induction of the hexosamine pathway and alterations in lipid metabolism were unique to GVP exposure. These specific changes in biochemical profiles could be attributed to perturbations of the biochemical pathway(s) or to altered cellular uptake of specific biochemicals from culture media in the presence of WTPM or GVP.

908 CHARACTERIZATION OF A WHOLE SMOKE *IN VITRO* EXPOSURE SYSTEM, THE MIMIC SMOKER BURGHART -01 (MSB-01).

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In vitro systems are frequently used to study cigarette smoke (CS)-induced lung injury. Traditional methods of exposure capture CS particulate phase with a filter pad or bubbling of the CS through PBS (sbPBS) or cell culture media. These exposure methods ignore potential interactions between CS gas and particulate phase. In order to better understand the effect of CS on the human airway, in vitro whole smoke exposure systems that utilize freshly prepared whole smoke are needed. Here we report on the characterization of a new in vitro whole smoke exposure system, the Burghart Mimic Smoker-01 (MSB-01, Heinrich Burghart Tabatechnik, GmbH). Using the MSB-01, BEAS-2B cells were seeded and exposed in 96-well plates. This eliminates the necessity for adaptive cultivation of cells to culture inserts used with other systems. Simultaneous delivery of CS to each well of the plate is achieved via a smoke distribution device located over the multiwell plate. Intraday and interday variability for particle delivery at three different dilution ratios as well as cytotoxicity measurements of BEAS-2B cells were determined. In general, intraday and interday variations for particulate deposition were less than 5% and 10% respectively at all dilutions tested, while variation of the EC50 measurements was approximately 13% intraday and 20% interday. Overall, the MSB-01 reproducibly delivered mainstream cigarette smoke in a dose dependent manner as well as equally across the multiwell plates. Therefore the MSB-01 in vitro CS exposure system provides a unique whole smoke exposure setup capable of high throughput functionality.

909 IMPROVEMENT OF MONKEY (MACACA FASCICULARIS) HEMATOPOIETIC CLONOGENIC ASSAYS FOR TOXICOLOGICAL STUDIES.

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The aim of this work was to improve haematopoietic clonogenic, assays for toxicological study in monkeys (Macaca fascicularis). Several protocols for bone marrow cells harvest, mononuclear cells cryopreservation, thawed and culture of monkey haematopoietic progenitors (number of cells seeded, duration of incubation, culture mecium, cytokines) have been tested.

In order to used this model in toxicology, the cytotoxicity of solvents classically used in in vitro test have been evaluated to determine no cytotoxic concentrations. T-2 toxin, a mycotoxin known to be myelotoxic, and well studied in our laboratory on rat, mouse, and human haematopoietic cells culture, has been used as referent myelotoxic molecule to improve the application of clonogenic assays of monkey haematopoietic progenitors to in vitro myelotoxicological studies. Standard operated procedure defined par ECVAM for human cells have been used.

Results show that there is a difference of sensitivity to T-2 toxin between several species tested. T-2 toxin is less cytotoxic for monkeys haematopoietic progenitors (10-6 M) than for human (10-7 M) and rat (10-8 M). Unfortunately, there is no in vivo data in monkey for this toxin. Consequently, it is not possible to perform an in vivo/in vitro correlation.

However, data show that this test is sensitive, specific, and reproducible. Its application to drug in development is in progress.

910 INTEGRATED RISK INFORMATION SYSTEM HEALTH ASSESSMENT OF PBDE CONGENERS.

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Polybrominated diphenyl ethers (PBDEs) have been increasingly identified in human biological media and several environmental compartments such as air, dust, and foods. The U.S. EPA has prepared Toxicological Reviews for four of the PBDE congeners: tetraBDE-47, pentaBDE-99, hexaBDE-153 and decaBDE-209. These four congeners are those for which toxicological studies suitable for dose-response assessments were available and are among the ones most commonly found in the environment and human biological media. The Toxicological Reviews underwent internal Agency review by EPA Offices and Regions, OMB and other interagency

reviews (ATSDR, NIEHS, and CPSC), public review, and external peer review by a panel of experts. An electronic version of the draft Toxicological Reviews and charges to the panel of experts is available.
Draft RfDs have been proposed for tetraBDE-47 (0.1 μg/kg-day), pentaBDE-99 (0.1 μg/kg-day), hexaBDE-153 (0.2 μg/kg-day) and decaBDE-209 (7 μg/kg-day), on the basis of neurodevelopmental effects observed in adult rodents after exposure to PBDE congeners during the neonatal period. For various reasons, the overall confidence in each of the four RfD assessments is considered "Low". There was inadequate information to assess the carcinogenic potential of BDE-47, BDE-99, and BDE-153. There was "suggestive evidence of carcinogenic potential" for BDE-209. The draft proposed oral cancer slope factor derived on the basis of chronic carcinogenicity studies of BDE-209 in rats and mice (National Toxicology Program, 1987) is 7 × 10–7 per μg/kg-day. Based on this cancer slope factor, the dose associated with an excess cancer risk of 10–5 is 14 μg/kg-day, i.e. greater than the RfD of 7 μg/kg-day for decaBDE. The proposed RfDs for the four PBDE congeners and cancer slope factor for BDE-209 should be considered drafts until such time the documents are finalized by the Agency. [The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA.]

911 RECENT DEVELOPMENTS IN THE U.S. EPA'S INTEGRATED RISK INFORMATION SYSTEM (IRIS) PROGRAM.

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IRIS is an EPA program containing consensus scientific positions on potential adverse human health effects that may result from chronic (or lifetime) exposure, or in select cases less-than-lifetime exposures, to chemicals in the environment. IRIS currently provides health effects information on over 540 chemical substances. IRIS contains chemical-specific summaries of qualitative and quantitative health information in support of two steps of the risk assessment process, i.e., hazard identification and dose-response evaluation. IRIS information includes a reference dose for non-cancer health effects resulting from oral exposure (the RfD), a reference concentration for non-cancer health effects resulting from inhalation exposure (the RfC), and a qualitative and quantitative assessment of carcinogenicity for both oral and inhalation exposures. Combined with specific situational exposure assessment information, the health hazard information in IRIS may be used as a source in evaluating potential public health risks from environmental contaminants. The program is extensively used by EPA Program and Regional Offices, other Federal agencies, state and local government agencies, international agencies, and the public including academia, regulated industries, environmental organizations, and individuals. In recent years the IRIS program has undergone a number of developments to improve the quality and the transparency of the IRIS. Some of the major improvements are: 1. Implementation of changes to the IRIS process, including the addition of Interagency review prior to external peer review, 2. Pilot completed for one of the IRIS assessments of less-than-lifetime exposure durations, 3. Enhanced uncertainty analysis, and 4. Initiation of a contract with National Academies of Science (NAS) to consider complex risk assessment issues arising in IRIS assessments. The views expressed in this poster are those of the authors and do not necessarily reflect the view or policies of the U.S. EPA.

912 CURRENT CHALLENGES FOR CARCINOGEN RISK ASSESSMENT.

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U.S. EPA's Integrated Risk Information System (IRIS) Program provides hazard identification and quantitative dose-response assessments for use in EPA chemical risk assessments. Implementation of EPA's 2005 Guidelines for Carcinogen Risk Assessment and Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure has presented several scientific challenges. Many assessments currently under development address complex issues including the interpretation of animal mode-of-action (MOA) data and its relevance to humans, selection of a weight-of-evidence (WOE) descriptor for carcinogenic potential, selection of low-dose extrapolation methods, and consideration of early-life exposures. An issue in applying MOA information is determining how much data are needed regarding key events to support qualitative and quantitative decisions. Analysis of cancer data for assessments under development has identified challenges associated with selecting a particular cancer descriptor for a range of chemicals with highly variable data sets. A WOE approach is proposed which includes characterization of the available data and the uncertainty arising from the attempt to assign chemicals to one of five descriptors lying on a continuum from "carcinogenic to humans" to "not likely to be carcinogenic to humans." Because EPA guidance calls for application of age-dependent adjustment factors when a mutagenic MOA is determined, decisions on the mutagenicity of a chemical impact the quantitative assessment. Assessing the mutagenic potential of chemicals that have some positive data for mutagenicity but may lack key evidence for a mutagenic MOA is a particular challenge. Decisions related to these issues cut across the scientific community and may impact risk assessments and rule-making. (The views expressed in this abstract are those of the authors and do not reflect the policies of the U.S. EPA.)

913 UNDERSTANDING MODE OF ACTION DATA AND UNCERTAINTY IN THE CHARACTERIZATION OF CANCER RISK ASSOCIATED WITH NAPHTHALENE-INDUCED TOXICITY.

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U.S. EPA's Integrated Risk Information System (IRIS) Program is undertaking a health assessment for naphthalene (NP). Consideration of the uncertainty associated with NP-induced toxicity is a key component of the analysis. Nasal and lung tumors have been observed in rats and mice, respectively, following inhalation exposure to NP. Numerous studies on the mode of action (MOA) of carcinogenicity in mouse lung are available, but less is known about the MOA in rat nasal tissue. The MOA analysis includes the putative roles of genotoxic effects, DNA adduct formation, cytotoxicity, oxidative stress, and glutathione-S-transferase (GST) induction. NP is mostly negative in classic genotoxicity batteries. Chromosomal damage, inferred from positive clastogenic responses, and NP-induced DNA adduct formation have been observed in vitro but have not been detected in vivo. The available assays may not have been performed under conditions that would provide for the formation of putative reactive metabolites and the potential for mutagenicity in nasal tissues has not been assessed. Exposure to NP causes cytotoxicity in animals which may contribute to carcinogenesis. Both rats and mice exhibit nasal cytotoxicity, while only rats develop tumors, and dose-response relationships have not been characterized at exposure levels below 10 ppm. Oxidative stress may play a role in the observed carcinogenesis, as the formation of activated metabolites has been shown to lead to lipid peroxidation products and NP tolerance has been associated with maintenance of the glutathione pool in mouse lung. The induction of hepatic GST isoforms in mouse lung has also been associated with the development of tolerance to NP. Analysis of the MOA data via a weight-of-evidence approach will inform the cancer assessment and the characterization of uncertainty. The views expressed are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA.

914 ADDRESSING UNCERTAINTY IN THE ESTIMATION OF HUMAN RISKS FROM TETRACHLOROETHYLENE EXPOSURE.

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An ongoing EPA human health risk assessment assesses the likelihood that tetrachloroethylene (TTCE) is carcinogenic to humans, based on: 1) its carcinogenicity in all lifetime rodent bioassay datasets, by both oral and inhalation routes of exposure; 2) the hepatocarcinogenicity of TTCE oxidative metabolites in rodents; 3) the reasonable assumption that the animal evidence indicates potential human cancer hazard; and 4) the consistent association of human TTCE exposure with excess risks for a number of cancers, although a causal association has yet to be definitively established. Accordingly, cancer dose-response analysis was undertaken. The cancer assessment also systematically weighs the impacts of a number of sources of uncertainty on the risk estimate(s), some of which can only be qualitatively explored (e.g., identification of mode(s) of action, human sensitivity and variability), while for others the potential quantitative impact on the risk estimate is examined (e.g., pharmacokinetic variability, statistical uncertainty in estimating points of departure, choice of tumor site). In general, cancer risk estimates aim to provide a reasonable upper bound on risk, here through choice of tumor type, point of departure, and low-dose extrapolation approach. In this case, however, a range of TTCE cancer values is calculated using several PBPK models, reflecting different plausible sources of sensitive metabolic parameters. In addition to carcinogenicity, TTCE exhibits numerous non-cancer health effects (e.g., neurotoxicity and liver, kidney, and developmental toxicity). Reference values (RfVs) based on a sensitive effect (neurotoxicity in humans) were compared with RfVs based on other effects, illustrating

dose dependency of the multiple observed toxicities. The non-cancer assessment also considers uncertainty based on extrapolation from laboratory animals to humans, human variability, and database deficiencies.
Disclaimer: The views expressed are those of the authors and not the US EPA.

915 A COMPARISON OF ANIMAL AND HUMAN DATA USED IN DERIVING A CARBON TETRACHLORIDE REFERENCE CONCENTRATION.

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EPA's IRIS Program is preparing a health assessment for carbon tetrachloride. An analysis was performed that compares the point of departure (POD) for a carbon tetrachloride reference concentration derived from a study of hepatic effects in rats to one derived from a study of hepatic enzyme levels in humans. The animal data come from a 2-year inhalation bioassay (Nagano et al., Inhal Toxicol, in press). Carbon tetrachloride induced liver toxicity in rats at ≥25 ppm. The POD based on rat data was derived using benchmark dose (BMD) modeling methods and 10% extra risk of fatty liver as the benchmark response. Physiologically based pharmacokinetic modeling was applied to yield a BMDL10[HEC] of 13 mg/m3 (expressed as a human equivalent concentration assuming continuous exposure). The human data come from a study of 135 workers occupationally exposed to carbon tetrachloride and 276 unexposed workers (Tomenson et al., Occup Environ Med 1995;52:508-14). Exposure assessment was based on historical personal monitoring data for various jobs at the plants and analysis of variance was used to investigate the relationship between carbon tetrachloride exposure and serum chemistry variables, controlling for age, sampling phase, and alcohol consumption. In humans, no effects on serum enzyme levels were seen in the low exposure category (≤6.3 mg/m3). Results for alkaline phosphatase and γ-glutamyl transferase suggest an exposure-related effect in the medium/high exposure categories (weighted average concentration = 35 mg/m3), which we considered an estimate of the lowest-observed-adverse-effect level (LOAEL). This is equivalent to 12.5 mg/m3 adjusted to continuous exposure. The similarity of the BMDL10[HEC] based on fatty change in rat liver and the LOAEL based on hepatic enzyme changes in humans supports the use of bioassay findings for establishing acceptable human exposures.
Disclaimer: The views expressed in this abstract are those of the authors and do not represent the policy of the U.S. Environmental Protection Agency.

916 RATS, MONKEYS, AND HUMANS: COMPARING POINTS OF DEPARTURE FOR USE IN THE DERIVATION OF A REFERENCE CONCENTRATION FOR 2-HEXANONE.

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U.S. EPA's Integrated Risk Information System (IRIS) is currently developing a health assessment for 2-hexanone (CASRN 591-78-6), as the potential for human exposure exists at Superfund sites. Thus, there is a need to develop an exposure estimate that is likely to be without an appreciable risk to humans. A review of both animal and human studies revealed that peripheral neuropathy is the critical effect following exposure to 2-hexanone. Thus, both animal and human data were considered for use in deriving a point of departure (POD), i.e., a dose from which the reference concentration (RfC) is derived. Decreased motor nerve conduction velocity (MCV) in the sciatic-tibial and ulnar nerves in male rats and monkeys, exposed to 2-hexanone via inhalation for six months, was analyzed using benchmark dose (BMD) methods, and PODs derived. The PODs were comparable across endpoints and species with the exception of the POD derived from ulnar MCV in rats, which was three to four times lower than PODs based on the other endpoints. In humans, data on the incidence of peripheral neuropathy in workers, as a function of 2-hexanone air levels in a coated fabrics plant, were analyzed using BMD methods, and the resulting POD was compared with the animal-derived PODs. The human-derived POD was an order of magnitude lower than the POD based on ulnar MCV in rats. However, this lower POD may have been due to the fact that the BMD analysis assumed 2-hexanone exposure occurred only via inhalation, while workers were also exposed via the oral and dermal routes. Furthermore, these workers were also co-exposed to methyl ethyl ketone, which can potentiate the toxicity of 2-hexanone. Because of these uncertainties in the human data, a POD based on the animal data is thought to be the best choice for evaluating the neurotoxicity of 2-hexanone exposure.
[The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA.]

917 EVALUATION OF TOXICITY ASSOCIATED WITH ORAL EXPOSURE TO CHLORDECONE: COMPARISON OF POINTS OF DEPARTURE FOR USE IN THE DERIVATION OF AN ORAL REFERENCE DOSE (RFD).

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Chlordecone (Kepone) is a chlorinated pesticide that was used primarily as an insecticide starting in the 1950's. The registration for chlordecone was canceled in 1976 after the intoxication of employees at a production facility in Hopewell, Virginia. Due to the persistence of chlordecone in the environment, the IRIS program is currently undertaking an assessment of chlordecone to develop a reference level for human exposure. The database for chlordecone includes limited human data from observational studies of occupationally exposed workers. Effects reported in over half of the workers included neurological effects (i.e., tremors, headaches and nervousness). Hepatomegaly, oligospermia and skin rashes were also observed in smaller subsets of these workers. The database for chlordecone also includes subchronic and chronic dietary exposure studies in laboratory animals. The primary noncancer effects from oral exposure to chlordecone in animals include lesions in the liver, kidney and testis; neurological effects (i.e., tremors); and alterations in sperm parameters and reduced fertility. The observations of kidney, liver, and testicular lesions along with sperm effects occur at similar dose levels. In the current IRIS assessment, these endpoints were compared qualitatively for biological significance and consistency across the database. Additionally, benchmark dose (BMD) modeling was used to derive points of departure (PODs) from these endpoints. The PODs were all within one order of magnitude of one another (from 0.12 to 0.3 mg/kg-day). After consideration of all endpoints, the increased incidence of kidney lesions in female rats was determined to be the most sensitive. Studies in both rats and mice support findings of kidney effects with chlordecone exposure.
[The views expressed in this abstract are those of the author and do not necessarily reflect the views or policies of the U.S. EPA]

918 U.S. EPA INTEGRATED RISK INFORMATION SYSTEM (IRIS) REASSESSMENT OF 1, 1, 2, 2-TETRACHLOROETHANE.

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The IRIS Program is updating the human health assessment for 1,1,2,2-tetrachloroethane (1122TCE). This chemical was nominated for inclusion in IRIS by the EPA Office of Air and Radiation. 1122TCE has been used as a solvent, a metal degreaser, and as an ingredient in paint removers, varnishes, and lacquers. 1122TCE has been found at 46 National Priority List sites according to EPA's Superfund Program. Human studies on 1122TCE are limited primarily to case reports describing acute oral and inhalation exposures to this chemical. These exposures elicit CNS effects (i.e., narcosis) along with gross congestion of thoracic organs and histological effects in the lungs, liver, and/or kidneys. High-dose exposures to 1122TCE can also lead to unconsciousness, respiratory failure, and death. Experimental animals have exhibited similar responses including hepatotoxicity (i.e., increased liver weight, enzyme levels, necrosis, and elevated bile acids), spleen pigmentation, reduced weight gain, and increased fetal resorptions. These data suggest that the primary target of 1122TCE-induced toxicity is the liver. In this reassessment, both non-cancer oral reference values (short-term, subchronic, and chronic) and cancer risk estimates were derived. The Benchmark Dose or the NOAEL/LOAEL approach was used to determine the potential points of departure (PODs) for deriving toxicity values for 1122TCE. PODs were determined for decreased body weight, increased liver weight, spleen pigmentation, hepatocellular necrosis, increased serum ALT and SDH, and decreased fetal weight. These PODs were within an order of magnitude of each other. A route-to-route extrapolation was considered for derivation of inhalation toxicity values from oral data. The toxicity values in the health reassessment for 1122TCE may be combined with exposure information to evaluate public health risks for 1122TCE at sites of concern.
Views expressed are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA.

919 U.S. EPA INTEGRATED RISK INFORMATION SYSTEM (IRIS) ASSESSMENT OF CERIUM OXIDE AND CERIUM COMPOUNDS.

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The IRIS Program is currently developing a health assessment for cerium oxide and cerium compounds. Cerium is used as a fuel-borne additive, polishing agent (for glass mirrors, plate glass, television tubes, ophthalmic lenses, and precision optics), glass constituent to prevent solarization and discoloration, and catalyst for automobile emission control systems. The database of inhalation studies for cerium in-

cludes human data from case reports of occupationally exposed workers and a subchronic cerium oxide inhalation study in Sprague-Dawley rats. Available human data describe workers who developed pneumoconiosis associated with the accumulation of cerium in the lungs after prolonged occupational exposure to cerium fumes or dust. The single oral subchronic study in rats observed pulmonary effects including increased lung weight and increased incidence of lymphoid hyperplasia, alveolar epithelial hyperplasia and pigment accumulation in the lung, and increased incidence of lymphoid hyperplasia and pigment accumulation in the bronchial lymph nodes. The human and animal data suggest that the lung is the primary target of toxicity following inhalation exposure to cerium oxide. The proposed mode of action for cerium-induced toxicity is the overloading of the pulmonary alveolar macrophages by cerium, leading to the release of inflammatory cytokines and fibrogenic growth factors and subsequent cell damage. A Reference Concentration was derived based on the pulmonary effects observed in the lungs of the rats, and a qualitative cancer assessment was developed. The information presented in the health assessment for cerium may be combined with exposure assessment information to evaluate potential public health risks from cerium exposure.
The views expressed in this poster are those of the authors and do not necessarily reflect the view or policies of the U.S. EPA.

920 MICROCYSTIN-LR: SUGGESTIVE EVIDENCE OF CARCINOGENICITY.

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Microcystins are monocyclic heptapeptide toxins produced by several species of cyanobacteria (blue-green algae). The primary mechanism of action of microcystins involves the inhibition of serine/threonine protein phosphatases PP1 and PP2A leading to hyperphosphorylation of cytosolic and cytoskeletal proteins and the subsequent disruption of cellular architecture. Several human epidemiological studies have reported an association between consumption of drinking water containing microcystins and liver or colon cancer in certain areas of China though no clear correlation with microcystin exposure was determined. Microcystin-LR (MCLR) is one of the most frequently detected cyanobacterial toxins, and one of the most toxic. Studies in rodents have indicated that MCLR induces promotion of preneoplastic foci in liver and colon and several in vivo and in vitro studies support plausible tumor promotion activity of the toxin. MCLR has been shown to modulate the expression of certain oncogenes, early-response genes and tumor necrosis factor-α and has been shown to affect cell division, cell survival and apoptosis. There has been one limited study of the oral carcinogenicity of MCLR; it was reported that chronic gavage doses of MCLR over 28 weeks failed to induce neoplastic nodules of the liver in mice. In 2006, IARC categorized MCLR as possibly carcinogenic to humans (Group 2B) based on the available epidemiologic information and supporting data. Applying the 2005 US EPA Guidelines for Carcinogen Risk Assessment there is "suggestive evidence of carcinogenic potential" of MCLR by the oral route of exposure. This is a unique situation where a compound lacking both strong epidemiological data and a positive chronic animal bioassay result has been found to have "suggestive evidence of carcinogenic potential."

921 CONSIDERATION OF THERAPEUTIC AND TOXICITY DATA FOR ORAL COBALT ASSESSMENT.

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Cobalt is widely distributed in the environment in low concentrations. An average person consumes about 11 micrograms of cobalt a day in their diet, and cyanocobalamin (vitamin B12) constitutes about half of daily intake of cobalt. Cobalt stimulates production of red blood cells through increased production of erythropoietin; thus has been used in the treatment of anemia. In short-term studies (Davis and Field, 1958), reversible polycythemia (increased red cells and hemoglobin levels) has been reported in healthy humans treated with 0.97 mg cobalt/kg-day. Therapeutically, similar doses (1 mg cobalt/kg-day) have been reported to cause reduced iodine uptake in euthyroid patients. However, long-term exposure in anemic children to higher doses of cobalt (2-4 mg/kg-day) has been reported to cause goiter. Similarly, long-term exposure (26 mg cobalt/kg-day for 15-45 days) of mice has been reported to cause severe thyroid toxicity. Exposure to levels of cobalt normally found in the environment is not harmful to humans and animals (ATSDR, 2004). However, use of cobalt in various manufacturing processes has resulted in contamination of ground water and soil at several sites. As a result, it is unclear if long-term human exposure to cobalt levels within an apparent therapeutic range would lead to adverse effects. Therefore, consideration of both polycythemia and decreased thyroid iodine uptake as possible critical endpoints for development of oral toxicity values in an assessment of risk for oral cobalt exposure is paramount. In this report, analysis of data indicates assessment based on thyroid effects will provide protection from adverse effects including polycythemia. (The report does not constitute views and policies of the U.S. EPA).

922 SCREENING-LEVEL HAZARD CHARACTERIZATION OF HIGH PRODUCTION VOLUME CHEMICALS.

M. Sonawane, T. Henry, M. Townsend and O. Hernandez. *U.S. EPA, Washington, DC.* Sponsor: L. Scarano.

The High Production Volume (HPV) Challenge Program is a voluntary initiative aimed at making publicly available screening-level health and environmental effects information on chemicals manufactured in or imported into the United States in quantities greater than one million pounds per year. Producers and importers of HPV chemicals voluntarily submitted previously unpublished information based on an internationally accepted battery of screening-level tests called the OECD Screening Information Data Set (SIDS).
OPPT is using a two-tiered approach to perform its hazard characterization. Tier 1 is a computerized sorting process whereby key elements of the submitted data set are compared to established criteria to prioritize chemicals for review. Tier 2 consists of developing a screening-level hazard characterization based upon an objective evaluation of the quality and completeness of the data provided in the Challenge Program submissions Many submitters grouped chemicals into categories. OPPT evaluates the submitted hazard information on individual chemicals and categories in accordance with established EPA and OECD guidance. Additional/updated hazard information of which EPA, sponsors or other parties have become aware is also included. As of October, 2007, over 100 hazard characterizations have been posted on the OPPT HPV website (http://www.epa.gov/chemrtk/hpvis/abouthc.htm).
The screening-level hazard characterizations do not evaluate the potential risks of a chemical or a chemical category, but will serve as a starting point for such reviews. OPPT will develop the screening-level risk characterizations, using the screening-level hazard characterization and available exposure information, as part of a risk-based decision-making process on HPV chemicals to support judgments concerning the need, if any, for further action.

923 DEVELOPMENT OF RISK CHARACTERIZATIONS UNDER THE U.S. HIGH PRODUCTION VOLUME (HPV) CHALLENGE PROGRAM.

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The HPV Challenge Program is a voluntary initiative designed to make publicly available screening-level health and environmental effects information on chemicals manufactured in or imported into the U.S. in quantities greater than one million pounds/year. In this Program, producers and importers identify and assess the adequacy of existing toxicity information and commit to conduct new testing if such data do not exist. The information consists of data on 18 internationally agreed to endpoints that are screening-level indicators of potential hazards (toxicity) for humans or the environment and environmental fate.
The U.S. EPA's Office of Pollution Prevention and Toxics (OPPT) is evaluating the data submitted in the HPV Challenge Program. OPPT developed a screening-level hazard characterization that consists of an objective evaluation of the quality and completeness of the data submitted by industry in accordance with established EPA guidance. In 2006 and 2007, EPA received data on uses and exposure-related data for chemicals on the Toxic Substances Control Act (TSCA) Inventory of existing chemicals, submitted in accordance with the requirements of the Inventory Update Reporting (IUR) rule. For the qualitative screening-level risk characterization documents, the exposure information has been combined with the screening-level hazard characterizations to develop this qualitative screening-level risk characterization. The purpose of the qualitative screening-level risk characterizations is two-fold: to identify data needs for individual chemicals or chemical categories and to inform risk management decisions aimed at reducing potential risks as part of OPPT's existing chemicals management process.
OPPT has recently completed its first round of risk characterization documents, which will be posted onto the EPA website in 2008. This poster presents examples of the qualitative risk assessment documents and an overview of the process of qualitative risk characterization within the framework of the HPV Challenge Program.

924 DATA FOR THE DEVELOPMENT OF DRINKING WATER HEALTH ADVISORY FOR N-NITROSODIMETHYLAMINE.

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N-Nitrosodimethylamine (NDMA) is a by-product of drinking water disinfection. It is released to the environment manufacturing processes involving alkyl amines and occurs in processed foods and in non-food consumer products; and produced endogenously in the gastrointestinal tract from nitrites and secondary amines from foods. In humans, acute exposures to NDMA have resulted in gastrointestinal (GI) disturbances, headaches; fever; enlarged liver; jaundice; and reduced function of the

liver, kidney, and lungs. In laboratory animals, NDMA, primarily, affects the liver and immune system. Although NDMA is fetotoxic, it is not teratogenic. Based on hepatotoxic effects in rats, a One-Day Health Advisory (HA) could be calculated as 0.2 mg/L for a 10 kg child, and the Ten-Day HA would be 0.1 mg/L. Based on hepatotoxic effects in mink, the Longer-Term HA for a 10 kg child can be calculated as 0.003 mg/L. Based on fetotoxic effects in mice, the Longer-Term HA for adults would be 0.002 mg/L. No Lifetime HA would be recommended or calculated because NDMA is carcinogenic; NDMA is also mutagenic. Based on the available animal and epidemiological data, NDMA is considered a likely human carcinogen, according to current EPA cancer guidelines. An oral slope factor that could be derived is 27 (mg/kg)/day)-1, yielding a unit risk of 7.7 × 10-4/(μg/L). Concentrations of NDMA in drinking water associated with lifetime cancer risks of 10-4, 10-5, and 10-6 can be calculated as 1.3 × 10-1, 1.3 × 10-2, and 1.3 × 10-3 μg/L, respectively. Allowing for increased susceptibility of neonates, the adjusted total lifetime cancer risks associated with NDMA drinking water concentrations of 1.3 × 10-1, 1.3 × 10-2, 1.3 × 10-3 μg/L would be 2 × 10-4, 2 × 10-5, and 2 × 10-6, respectively, respectively.
[The opinions herein are those of the author and do not necessarily reflect the opinions of the U.S. Environmental Protection Agency (USEPA)]

925 PROVISIONAL ADVISORY LEVEL (PAL) DEVELOPMENT FOR HCL AND HBR.

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PAL values developed for hazardous materials by the U.S. EPA represent general public emergency exposure limits for oral and inhalation exposures corresponding to three different severity levels (1, 2, and 3) for 24-hour, 30-day, 90-day, and 2-year durations. PAL 1 represents the threshold for mild effects; PAL 2 represents the threshold for serious, irreversible or escape-impairing effects; PAL 3 represents the threshold for lethal effects. PALS have not been promulgated nor have they been formally issued as regulatory guidance. They are intended to be used at the discretion of risk managers in emergency situations when site specific risk assessments are not available. Application of PAL protocols was performed for hydrogen chloride and hydrogen bromide, as experimental data permitted. The experimental data sets for both chemicals included limited inhalation studies; oral data were available only for HCl. A contributing factor to the oral toxicity of HCl is pH due to the possibility of acid burns.
Oral 24-hr PALs for HCl are: PAL 1 = 250 mg/L (pH 3.0); PAL 2 = 420 mg/L (pH 2.0); and PAL 3 = NR (not recommended) (pH 1.0). Data were insufficient to derive 30-d, 90-d, or 2-yr oral PALs for HCl. HCl inhalation PAL values for 24 hr exposure are: PAL 1 = 1.8 ppm; PAL 2 = 10 ppm; and PAL 3 = 35 ppm; the 30 day and 90 day inhalation exposure values are PAL 1 = 0.90 ppm and PAL 2 = 3.3 ppm; the 2 yr inhalation exposure, PAL 1 = 0.18 ppm. Data were insufficient for derivation of the 2-yr PAL 2 and the 30-d, 90-d, and 2-yr PAL 3 for HCl. Data were insufficient to derive oral PALs for HBr. HBr inhalation PAL values for 24 hr exposure are: PAL 1 = 1.0 ppm; PAL 2 = 2.1 ppm; and PAL 3 = 8.2 ppm. Data were insufficient for derivation of the 30-d, 90-d, and 2-yr PAL 1, 2, and 3 for HBr. (This abstract presents PAL values that are subject to change pending further review.)

926 PROVISIONAL ADVISORY LEVELS (PALS) FOR RED PHOSPHORUS SMOKE.

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PAL values developed for hazardous materials by the U.S. EPA represent general public emergency exposure limits for oral and inhalation exposures corresponding to three different severity levels (1, 2, and 3) for 24-hour, 30-day, 90-day, and 2-year durations. PAL 1 represents the threshold for mild effects; PAL 2 represents the threshold for serious, irreversible or escape-impairing effects; PAL 3 represents the threshold for lethal effects. PALS have not been promulgated nor have they been formally issued as regulatory guidance. They are intended to be used at the discretion of risk managers in emergency situations when site specific risk assessments are not available. The PAL protocol has been applied to estimate inhalation exposure limits for red phosphorus (RP) smoke; exposure limits for oral exposure to RP smoke are not developed because of the lack of data.
RP smoke, which is produced by the combustion of RP/butyl rubber (RP/BR), is used as a military smoke screen. It is made up of a complex mixture of phosphoric acids including o-phosphoric acid, a strong mineral acid with corrosive properties. RP/BR smoke is an irritant that affects the nasal cavity, larynx, trachea, and pulmonary regions of the respiratory tract in exposed animals. Human data for RP/BR smoke is limited; therefore, PAL estimates were based on evaluation of experimental data on rats, mice, guinea pigs, and rabbits.
PAL estimates for inhalation exposure to RP smoke will be presented: The 24-hour PAL values for severity levels 1, 2, and 3 are 3.8, 7.2, and 8.4 mg/m^3, respectively. The 30-day PAL values are 0.27, 0.96, and 4 mg/m^3, respectively. The 90-day PAL values are the same as the 30-day PAL: 0.27, 0.96, and 4 mg/m^3, respectively. Data are not available for developing 2-year inhalation PAL values for any severity level. (This abstract presents PAL values that are subject to change pending further review.)

927 PROVISIONAL ADVISORY LEVEL (PAL) DEVELOPMENT FOR PERFLUOROISOBUTYLENE (PFIB).

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PAL values developed for hazardous materials by the U.S. EPA represent general public emergency exposure limits for oral and inhalation exposures corresponding to three different severity levels (1, 2, and 3) for 24-hour, 30-day, 90-day, and 2-year durations. PAL 1 represents the threshold for mild effects; PAL 2 represents the threshold for serious, irreversible or escape-impairing effects; PAL 3 represents the threshold for lethal effects. PALS have not been promulgated nor have they been formally issued as regulatory guidance. They are intended to be used at the discretion of risk managers in emergency situations when site specific risk assessments are not available. Application of PAL protocols has been performed for perfluoroisobutylene (PFIB) to estimate inhalation exposure limits, to the degree supported by the available data. PFIB is a colorless gas that is usually an unwanted byproduct of the production of tetrafluoroethylene. The experimental data set for PFIB included extremely limited human data and numerous animal studies.
PAL estimates for PFIB will be presented. No oral PAL values are recommended because no data from oral exposure to humans or animals were found; PFIB decomposes in aqueous solutions so should not exist in concentrations of concern. Inhalation PAL values for a 24-hour exposure duration to PFIB are 0.0025 ppm (PAL-1), 0.0042 ppm (PAL-2) and 0.0091 ppm (PAL-3); for the 30-day exposure duration, the values are 0.0025 ppm (PAL-1) and 0.0042 ppm (PAL-2); for the 90-day exposure duration, the values are 0.00051 ppm (PAL-1) and 0.0042 ppm (PAL-2); and for the 2-year exposure duration, the values are 0.00051 ppm (PAL-1) and 0.0042 ppm (PAL-2). Because data are insufficient, PAL 3 estimates are not recommended for exposure durations of 30 days, 90 days and 2 years. (This abstract presents PAL values that are subject to change pending further review.)

928 PROVISIONAL ADVISORY LEVELS (PALs) DEVELOPMENT FOR POLYTETRAFLUOROETHYLENE (PTFE), TETRAFLUOROETHYLENE (TFE), AND CARBONYL DIFLUORIDE (COF_2).

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PAL values developed for hazardous materials by the U.S. EPA represent general public emergency exposure limits for oral and inhalation exposures corresponding to three different severity levels (1, 2, and 3) for 24-hr, 30-d, 90-d, and 2-yr durations. PAL 1 represents the threshold for mild effects; PAL 2 represents the threshold for serious, irreversible or escape-impairing effects; PAL 3 represents the threshold for lethal effects. PALs have not been promulgated nor have they been formally issued as regulatory guidance. They are intended to be used at the discretion of risk managers in emergency situations when site specific risk assessments are not available. Application of PAL protocols has been performed for PTFE, TFE, and COF_2 to estimate oral and inhalation exposure limits as experimental data permit. PTFE resins are used in nonstick coatings, plastics, and other products. Experimental data for PTFE showed that toxicity was due to decomposition products of heated (≥ 230°C) PTFE, including TFE and COF_2. The experimental data set for TFE was robust and included numerous animal studies. The experimental data set of COF_2 was marginally adequate, including few animal studies with limited data. Data gaps are identified.
Oral PAL estimates are not derived due to insufficient data. Inhalation PAL estimates for PTFE are not derived. Inhalation PAL estimates for TFE and COF_2 will be presented. Inhalation PAL values for 24-hr exposure to TFE are 50 ppm (PAL 1), 75 ppm (PAL 2), and 330 ppm (PAL 3). Inhalation PAL values for 30-d, 90-d, and 2-yr exposure to TFE are 4.6 ppm (PAL 1), 19 ppm (PAL 2), and 74 ppm (PAL 3). Inhalation PAL values for 24-hr exposure to COF_2 are 0.0087 ppm (PAL

1), 0.017 ppm (PAL 2), and 0.05 ppm (PAL 3). Data are insufficient to derive COF_2 PALs for longer exposure durations. (This abstract presents PAL values that are subject to change pending further review.)

929 PROVISIONAL ADVISORY LEVELS (PALS) FOR PHOSGENE.

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PAL values developed for hazardous materials by the U.S. EPA represent general public emergency exposure limits for oral and inhalation exposures corresponding to three different severity levels (1, 2, and 3) for 24-hour, 30-day, 90-day, and 2-year durations. PAL 1 represents the threshold for mild effects; PAL 2 represents the threshold for serious, irreversible or escape-impairing effects; PAL 3 represents the threshold for lethal effects. PALS have not been promulgated nor have they been formally issued as regulatory guidance. They are intended to be used at the discretion of risk managers in emergency situations when site specific risk assessments are not available. The PAL protocol was applied to estimate inhalation exposure limits for phosgene (CG); exposure limits for oral exposure to CG are not developed due to insufficient data.

Phosgene is a gas that has been used as a chemical warfare agent and is currently used as an intermediate in the polyurethane industry for the production of isocyanates. Although immediately upon exposure lacrimation and upper respiratory irritation can occur, the main effect in the target organ, a progressive pulmonary edema, occurs after a latency period of 1-24 hours. Human data for CG are limited; therefore, PAL estimates were based on evaluation of experimental data in rats.

PAL estimates for inhalation exposure to CG will be presented: The 24-hour PAL values for severity levels 1, 2, and 3 are 0.0017, 0.0033 and 0.022 ppm, respectively. The 30-day PAL values are 0.0006 and 0.0012 ppm for the PAL 1 and 2 values, respectively. These values were also accepted as the 90-day and 2-year PAL 1 and 2 values. Data were not available for deriving 30-day, 90-day and 2-year PAL 3 values. (This abstract presents PAL values that are subject to change pending further review.)

930 USE OF GENETIC TOXICITY TEST BATTERY IN HAZARD IDENTIFICATION FOR POTENTIAL CARCINOGENICITY OF VETERINARY DRUGS AND FEED INGREDIENTS.

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The DES proviso of the Delaney Clause of the FFD&C prohibits the use in food-producing animals of any compound that induces cancer in animals or humans unless it can be determined by an FDA approved analytical method that no residue of that compound will be found in edible tissues of these animals (21 CFR 500.80). Therefore, assessing carcinogenic potential of an animal drug or feed ingredient used in food-producing animals is one of the key components that CVM has been using for safety assessment of veterinary drug residues or feed ingredients in human food. CVM routinely uses a battery of genetic toxicity tests as the main tool for predicting carcinogenic potential of veterinary drugs or feed ingredients. Three standard genotoxicity tests recommended in the VICH Guideline GL 23 are: 1) a test for gene mutation in bacteria, 2) an in vitro test for chromosomal effects, gene mutations in mammalian cells, and 3) an in vivo test for chromosomal effects using rodent haematopoietic cells (usually micronucleus assay). Unequivocal negative results from genotoxicity testing are considered sufficient evidence of a lack of carcinogenic potential via a genotoxic mechanism. In general, chronic bioassays for oncogenicity in two rodent species are required for genetox positive compounds; however, CVM may consider on a case-by-case basis results from structure activity relationships and subchronic toxicity data for neoplasia when available, along with genetic toxicology data in determining the need for chronic bioassays for oncogenicity.

931 MINIMAL RISK LEVELS FOR ORGANIC ARSENICALS.

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Humans may be exposed to a variety of organic arsenicals (mainly methyl and phenyl derivatives of arsenic acid) used in agriculture. The preponderance of toxicity data for organic arsenicals involves oral exposure. It is generally accepted that the arsenic-carbon bond is quite strong and most mammalian species do not have the capacity to break this bond; thus inorganic arsenic is not formed during the metabolism of organic arsenicals. In most species, including humans, ingested (or exogenous) MMA(V) and DMA(V) undergo limited metabolism, do not readily enter the cell, and are primarily excreted unchanged in the urine. Human data are limited to case reports of individuals intentionally ingesting pesticides containing organic arsenicals. Symptoms of gastrointestinal irritation were consistently reported. Animal data have primarily focused on the toxicity of monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and roxasone. After reviewing the literature, an MRL of 0.1 mg/kg/day was derived for intermediate-duration oral exposure to MMA based on increased incidence of diarrhea in rats (Arnold et al. 2003; Crown et al. 1990) and an MRL of 0.01 mg/kg/day was derived for chronic-duration oral exposure to MMA based on increased incidence of progressive glomerulonephropathy in mice (Arnold et al. 2003; Gur et al. 1991). In addition, an MRL of 0.02 mg/kg/day was derived for chronic-duration oral exposure to DMA based on increased incidence of vacuolization of urinary bladder urothelium in mice (Arnold et al. 2006; Gur et al. 1989). Benchmark dose (BMD) analyses of the dose response data were conducted and the BMDL10 was used as point of departure to derive the three MRLs. These MRLs are presented and discussed in the ATSDR toxicological profile for arsenic released in October 2007. These substance-specific health guidance values are intended to serve as screening levels for use by health assessors and other responders to identify contaminants and potential health effects that may be of concern at hazardous waste sites.

932 ATSDR'S CHRONIC MINIMAL RISK LEVEL (MRL) FOR STYRENE.

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Styrene is a high production chemical used in the manufacture of polystyrene plastics and resins, pipes, automotive components, plastic drinking glasses, car tires, shoes, and polyester resins used as fiberglass reinforcement material. ATSDR has recently re-evaluated the toxicity of styrene and an updated toxicological profile for public comment was released in October 2007. Occupational exposure studies have firmly established the central nervous system as the critical target of toxicity. Both short- and long-term exposures to styrene can result in neurological effects including decreased color discrimination, vestibular effects, hearing impairment, symptoms of neurotoxicity, particularly "feeling drunk" and tiredness, delays in reaction time, impaired performance on attention and memory tests, and impaired nerve conduction velocity. The LOAELs for these effects range from 10 to 93 ppm. Two approaches to deriving a chronic-duration inhalation MRL for styrene were considered. The first approach involved deriving an MRL based on a single study identifying a sensitive LOAEL. Kishi et al. (2001) found a significant increase in color confusion index (CCI) in styrene workers exposed to 10 or 46 ppm; no effects were observed in workers exposed to 4 ppm. The second approach involved the use of a LOAEL estimated from a meta-analysis of occupational exposure studies finding effects on color vision and reaction time (Benignus et al. 2005). Significant linear relationships between choice reaction time and cumulative styrene exposure and CCI score and cumulative styrene exposure were found. It was estimated that exposure to 20 ppm for 8 work-years would result in a 6.5% increase in choice reaction time and a 2.23% increase in CCI score; 20 ppm was considered a LOAEL. The second approach was used to derive the MRL because it is based on a large number of subjects. The LOAEL of 20 ppm was divided by an uncertainty factor of 100 (10 for use of a LOAEL and 10 for human variability) resulting in a chronic-duration inhalation MRL of 0.2 ppm.

933 INHALATION MINIMAL RISK LEVELS (MRLS) FOR ETHYLBENZENE.

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Ethylbenzene is an organic solvent primarily used for the production of styrene and has a minor use in the manufacture of several other organic compounds. Effects following ethylbenzene exposure in humans include eye irritation, chest constriction, and neurological effects such as dizziness and vertigo. Animal studies have reported neurological, hepatic, respiratory, renal, and developmental effects. Acute and intermediate-duration animal studies suggest that the auditory system is a target of toxicity. Several volatile organic solvents have also been suspected of causing decreased hearing in humans and there have been confirming studies in animals exposed to toluene, styrene, and xylene. ATSDR derived acute and intermediate-duration inhalation MRLs of 10 ppm and 0.7 ppm, respectively, for ethylbenzene. The MRLs were based on neurophysiological outcomes and histology (loss of cochlear outer hair cells) in rats exposed to ethylbenzene vapor. A chronic-duration MRL of 0.3 ppm was derived based on nephropathy in female rats exposed to inhaled ethylbenzene in a 2 year NTP study.

934 AIR TOXICS HOT SPOTS PROGRAM RISK ASSESSMENT GUIDELINES, PART II: TECHNICAL SUPPORT DOCUMENT FOR DESCRIBING AVAILABLE CANCER POTENCY FACTORS.

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The Technical Support Document for Cancer Potency Factors (TSD) contains cancer unit risks and potency factors for 107 of the 201 carcinogenic substances or groups of substances for which emissions must be quantified under the requirements of the Air Toxics "Hot Spots" Information Act of 1987 (AB2588). The purpose of the TSD is to provide a summary of the data supporting the carcinogenic potential of the substance or group of substances and to provide the calculation procedure used to derive the estimated unit risk and cancer potency factors. In this document, the Office of Environmental Health Hazard Assessment (OEHHA) is responding to the requirements of the Children's Environmental Health Protection Act of 1999 (SB25) by revising the procedures for derivation and application of cancer potency factors to take account of general or chemical-specific considerations which suggest that children may be especially susceptible to certain carcinogenic effects. Although the existing cancer potencies will not be changed, the application of these values will be updated to reflect concerns associated with early-life exposures. These changes include the use of exposure weighting factors in calculating risks from exposures of infants, children and adolescents to reflect their anticipated special sensitivity to carcinogens. OEHHA will apply the default cancer potency factor age adjustments described above to all carcinogens unless data is available which allows for the development of chemical-specific cancer potency factor age adjustments. The TSD also describes the use of benchmark dose methodology for cancer potency estimation with an explicit choice of the method for low-dose extrapolation, either assuming low-dose linearity or, for certain carcinogens where data indicate that this is appropriate, a "margin of exposure" or safety/uncertainty factor based approach. Additionally, cancer potency and unit risk values and a data summary for ethyl benzene have been added to the TSD.

935 A REVIEW OF THE ROLE OF PEROXISOME PROLIFERATORS AS MEDIATIORS OF ENVIRONMENTAL TOXICANTS DURING DEVELOPMENT.

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Regulatory agencies rely on mechanistic data to evaluate issues such as biological plausibility for risk assessment. Data on peroxisome proliferator activated receptors (PPARs) relating to mediation of carcinogenesis in rodents are available, but the role of PPARs in developmental toxicity is unclear. Accordingly, we reviewed the literature for information on PPARs and their possible role during development. Topics/areas considered were: a) Expression of PPAR isoforms in reproductive tissues, placenta, embryo, and fetus, b) Conservation across mammalian species and function of these nuclear receptors during embryo-fetal development, c) Role of PPARs as mediators of toxic effects during development, d) Pharmacology of drugs targeting PPAR in therapeutic treatments for fertility troubles in humans, e) Developmental toxicity studies in PPAR knock-out animals and non-responding species when available, and f) Use of this information in developmental toxicity and risk assessment. The review indicated that unique considerations must be applied when using PPAR mechanistic data in developmental toxicity risk assessment.

936 ESTIMATING ACCEPTABLE LEVELS OF EXPOSURE TO AIRBORNE SENSORY IRRITANTS USING THE RD_{50}.

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Exposure to airborne sensory irritants may result in complaints and concerns from the public. However, relatively few acute Reference Exposure Levels (RELs) are available to assess potential public health impacts of exposure to airborne chemicals routinely emitted by industrial facilities in California. Two major reasons for the limited number of RELs are resources needed to derive the values and paucity of quantitative toxicological data for acute exposures with non-lethal endpoints. Thus, we investigated the availability of a quantitative measure that could be readily used to estimate an REL. The RD_{50}(exposure concentration capable of producing a 50% respiratory rate decrease in male mice) is a standardized method for measuring sensory irritation effects in mice. Studies have shown good correlations between the predicted levels from the RD_{50}s to Occupational Exposure Limits, Lowest Observed Adverse Effect Levels (LOAELs) in humans and acute RELs for the general public. We identified RD_{50}s from the literature for 20 of the chemicals identified under the California Air Toxics Hot Spots Program for which acute RELs were unavailable due to limited quantitative information, but for which sensory irritation was a likely concern. Several of these compounds are of interest since they are emitted in significant quantities from stationary point sources in California, are components of emissions from mobile sources (i.e. vehicle exhaust) or are generated by atmospheric reactions from other ambient air pollutants. Using the derived relationship between RD_{50}s and RELs, of $REL = 0.00026*RD_{50}^{1.4}$, we estimated acute RELs for these 20 compounds. Chronic RELs have already been derived for several of these compounds: in cases where the endpoint of the chronic REL is a respiratory tract effect the RD_{50}-derived acute REL is generally consistent with the basis and value of the chronic REL. The RD_{50} may thus be a useful measure upon which to base acute RELs.

937 AN APPROACH FOR TOLUENE CHRONIC AND 8-HOUR REFERENCE EXPOSURE LEVELS WITH BENCHMARK DOSE ANALYSIS FOR COLOR VISION IMPAIRMENT AND PBPK MODELS FOR ADULT-CHILD VARIABILITY.

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The Office of Environmental Health Hazard Assessment is in the process of revising our current approach to noncancer risk assessment to ensure that children's health is protected and to more fully utilize new methods. Risk assessment methodologies including benchmark dose analysis and PBPK modeling are employed to derive an 8-hour and an updated chronic Reference Exposure Levels (RELs) for toluene. There is evidence that color vision impairment occurs with lower chronic exposure concentrations than neurobehavioral deficits in workers and is thus the most sensitive endpoint. Using the data from a human worker study of Zavalic et al. (1998), a benchmark dose estimate for color vision impairment (45 mg/m3;12 ppm) was generated as the point of departure (95% lower confidence interval of the 5% response level) using a dichotomous modeling approach. To account for toxicokinetic variability among the human population, published PBPK data modeling the toluene blood AUC variability among several child age groupings and adults is used to develop a weighted adult-child factor of 1.5 that estimates the overall adult-child pharmacokinetic variability (ratio of the 95th percentile for children over the 50th percentile for adults). An additional standard intraspecies toxicodynamic uncertainty factor of 10 is also applied for greater child susceptibility to neurotoxic effects, resulting in a combined intraspecies uncertainty factor of 15. Following duration exposure adjustments, a possible chronic REL of 1 mg/m3 (0.3 ppm) and a new 8-hr REL of 5 mg/m3 (1 ppm) are proposed. An 8-hour REL would be useful for the assessment of the health impacts on children in schools, who would typically be exposed for up to 8 hours per day when school is in session. Application of the benchmark dose and PBPK techniques lowers the uncertainty associated with reference exposure levels and helps ensure the protection of infants and children.

938 DEVELOPMENT OF A CANCER POTENCY FACTOR FOR STYRENE.

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Styrene is produced at about 10 billion pounds per year in the US and used primarily to make rubber and plastics. The potential of styrene to cause cancer was evaluated after review of epidemiological studies, cancer bioassays, and other studies. We agreed with IARC that there is limited epidemiological evidence of carcinogenicity for styrene. A cancer potency factor of 0.0025 $(mg/k\text{-}d)^{-1}$ was estimated from the data on pancreatic cancer in Kogevinas et al. (1994). In animals, lung tumors were observed in female and male mice exposed by inhalation for their lifetime and in male mice exposed by gavage. Lung tumors developed in the progeny of mice exposed to styrene in utero and then after birth by gavage. Female rats developed mammary gland tumors when exposed by inhalation or drinking water ingestion. Styrene has given positive results in several genotoxicity tests including reverse mutation in Salmonella typhimurium and induction of DNA strand breaks and chromosomal aberrations in human and animal cells. We concluded that styrene is a carcinogen. The potency calculated for styrene is based on the inhalation study of Cruzan et al. (2001) in CD-1 mice which was a lifetime study and used an adequate number of animals (70/sex/exposure level) and several concentrations of styrene (20, 40, 80, and 160 ppm). The study provided adequate data to derive a dose response cancer slope for lung bronchioloalveolar adenomas, and is supported by several additional studies and lines of evidence concerning potential carcinogenic effects of styrene. The inhalation cancer potency of 0.026 $(mg/k\text{-}d)^{-1}$ is similar to the potency of other chemicals that contain a benzene ring. A health-protective (one in a million risk) concentration of 0.5 ppb for multi-route exposure to styrene in drinking water was derived.

939 SELENIUM - DEVELOPMENT OF A HEALTH PROTECTIVE CONCENTRATION IN DRINKING WATER.

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A risk assessment is being conducted for water-soluble and bioavailable selenium compounds for developing a health based value for updating the existing California drinking water standard. The chemical forms include the more prevalent selenate and selenite ions in water and selenomethionine and selenocysteine in dietary items. The health effects evaluation included consideration of toxicity, nutritional essentiality, and dietary, drinking water and nutritional supplement intakes, with a focus on toxicity vs. essentiality. The critical effect is selenosis following dietary intake from contaminated locally grown foods in China with a No-observed-effect level (NOEL) of 15 ug/kg-day. The signs included nail and hair changes, skin lesions, nervous system abnormalities, and vascular effects. Applying an uncertainty factor of three to the NOEL provided an acceptable daily dose of 350 ug/day for a body weight of 70 kg. Intake from other sources, such as the mean selenium dietary intake in the U.S. of 114 ug/day and a dietary plus supplement intake of 175.4 ug/day at the 90th percentile, would reduce the amount permissible in drinking water. Data also suggested that children and pregnant women with their fetuses may be more susceptible to the effects of selenium deficiency. Infants (0 to 6 mos) have a tolerable upper intake level of 45 ug/day and a standard powdered formula intake of 14 ug/day, based on the same concentration of 18 ug/L in human milk and in standard powdered formula (reconstituted) available commercially in the U.S. The assessment provided a health protective concentration in drinking water in the range of 25 to 40 ug/L. This is compared to the U.S. Environmental Protection Agency's maximum contaminant level of 50 ug/L and World Health Organization's guideline value of 10 ug/L.

940 CARBOFURAN: TOXICITY PROFILE AND NON-DIETARY RISK EVALUATION.

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The cholinergic insecticide carbofuran (CF) was subjected to acute, subchronic & chronic non-dietary health risk assessment. The acute LED_{05} of 0.01 mg/kg (ED_{05} = 0.02 mg/kg) was calculated from observations of abnormal chewing behavior in pregnant CD rats following gavage exposure. The subchronic / chronic NOEL of 0.1 mg/kg/day was based on testicular toxicity and bodyweight gain suppression at 0.2 mg/kg/day in Druckrey rats in a 60-day gavage study. Dermal and inhalation exposures for five occupational handler categories were estimated using the Pesticide Handlers Exposure Database; dip / slurry applicator estimates came from equations in the Risk Assessment Guidance for Superfund and from air saturation levels. Handlers: acute dermal ADDs, 2-6360 µg/kg/day (MOEs, <1-5); seasonal dermal ADDs, 0.6-2120 µg/kg/day (MOEs, <1-167); annual dermal ADDs, 0.1-354 µg/kg/day (MOEs, <1-1000); acute inhalation ADDs, 0.03-41 µg/kg/day (MOEs, <1-333); seasonal inhalation ADDs, 0.02-16 µg/kg/day (MOEs, 6-5000); annual inhalation ADDs, 0.01-3 µg/kg/day (MOEs, 33-10,000). Fieldworkers: acute dermal ADDs, 7-99 µg/kg/day (MOEs, <1-1); seasonal dermal ADDs, 0.9-70 µg/kg/day (MOEs, 1-111); annual dermal ADDs, 0.1-12 µg/kg/day (MOEs, 8-1000). Resident / bystander exposure scenarios were estimated using air monitoring in Imperial and Sacramento Counties. Ambient air: acute ADDs (infants), 0.0014-0.070 µg/kg/day (MOEs, 143-7143); (adults), 0.0007-0.034 µg/kg/day (MOEs, 294-14,286); seasonal ADDs (infants), 0.0004-0.020 µg/kg/day (MOEs, 5000-250,000); (adults), 0.0002-0.010 µg/kg/day (MOEs, 10,000-500,000); annual ADDs (infants), 0.0001-0.003 µg/kg/day (MOEs, 33,000-1,000,000); (adults), 0.00007-0.002 µg/kg/day (MOEs, 50,000-1,428,571). Application site air: 1-hr ADDs in infants & adults - 0.550 µg/kg & 0.099 µg/kg (MOEs, 18 & 101); 24-hr ADDs in infants & adults - 0.454 & 0.216 µg/kg (MOEs, 22 & 46). Potential health risks requiring mitigation are usually indicated when MOEs are below a target value of 100.

941 HUMAN HEALTH RISK ASSESSMENT FOR EXPOSURE TO POLYCHLORINATED BIPHENYLS IN CONTAMINATED BUILDINGS.

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ABSTRACT BODY: It is not uncommon to find polychlorinated biphenyls (PCBs) contamination inside industrial buildings, because of extensive use in the past, especially in transformers. Limited guidance is available for conducting human health risk assessment (HHRA) for exposure to contaminants on building surfaces rather than environmental media. By adopting exposure parameters from the few available scientific reports, this paper presents HHRAs performed on two buildings at a facility in California. Previous use and spills of PCBs were reported in Buildings A and B, resulting in surface contamination on flooring materials (concrete, wood, and asphalt) inside the buildings. Concrete chips, dust, wall wipes, and indoor air were sampled inside these buildings during site investigation. Maximum PCB levels of 69 ng/m^3 indoor air and 0.7 mg/kg in concrete chips were used in the HHRA for Building A. Since the proposed future use of Building A is residential, incidental ingestion and direct dermal contact with the contaminated floor were considered incomplete exposure pathways. The residential cancer risk estimate and non-cancer hazard index (HI) from inhalation of indoor air are 2E-5 and 0.9. The industrial worker cancer risk estimate and non-cancer HI from inhalation of indoor air are 1E-5 and 0.7. For the industrial Building B, maximum PCB levels were 19 ng/m^3 in indoor air and 105 mg/kg in concrete chips. Human exposure via inhalation of indoor air, direct dermal contact, and incidental ingestion of dust on the floor were evaluated. The cancer risk estimate and non-cancer HI are 1E-3 and 261 for residents and 4E-4 and 26 for industrial workers. Exposure via direct dermal contact to the floor contributes most of the cumulative risk and HI for Building B. Limited downward migration through the flooring materials and high lipophilicity of PCBs increase the significance of human exposure via direct dermal contact with the building surfaces and dust ingestion from hand-to-mouth activities.

942 RESIDENTIAL PESTICIDE STUDY: EVALUATION OF RESIDUAL ORGANOCHLORINE PESTICIDES AROUND RESIDENTIAL PROPERTIES IN CALIFORNIA.

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Chlordane and other organochlorine pesticides (OCPs) were commonly used as termiticides around structures until 1988. Above-ground use of chlordane was phased out between 1978 and 1983 by the USEPA, although chlordane was used as a termiticide for wooden structures until it was prohibited in 1988. The California Department of Toxic Substances Control (DTSC) conducted a study in 2004 on these termiticides. In the DTSC Study, three proposed school sites, located in Los Angeles, San Diego and San Bernardino Counties, were selected for evaluation for the presence of OCPs. Each proposed school site had multiple residential properties. DTSC collected a total of 176 soil samples at three depths around building perimeters. Samples were analyzed for OCPs by a California certified laboratory using U. S. EPA method 8081A. OCPs were most frequently detected in the surface soil samples (0 to 0.5 feet below ground surface). The highest frequencies of detection of OCPs were chlordane (98 percent), DDT (95 percent), DDE (91 percent), and dieldrin (71 percent). Risk-screening evaluation results indicated elevated risks and hazards to human health at all three proposed school sites, associated primarily with chlordane and dieldrin in surface soils. Approximately 50 percent of the chlordane and dieldrin detections had an associated risk greater than 1 in a million (>1E-06), and approximately 20 percent of the chlordane and dieldrin detections had an associated risk greater than 1 in 100,000 (>1E-05). In addition, DTSC investigated OCPs at additional residential properties proposed for school sites in 18 California counties and found similar results. Based on results of the Study and investigation results at these additional proposed school sites, DTSC recommends sampling and analyses for OCPs be routinely conducted at proposed school sites where historically residential structures have been present.

943 FEXOFENADINE AS A PROBE DRUG FOR TRANSPORTER ACTIVITY: DEVELOPMENT AND VALIDATION OF AN HPLC-MS/MS METHOD.

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Membrane transporters are key gatekeepers, controlling disposition of chemicals. While the importance of membrane transporters is clear in absorption, distribution, metabolism, and excretion of xenobiotics, tools to investigate their specific roles in humans *in vivo* are needed. Fexofenadine, an approved, well-tolerated drug, is a promising probe drug for studies of transporter function in humans. Although fexofenadine pharmacokinetics are known to be controlled by transporters, the contributions of individual transporters are not fully elucidated. In order to measure fexofenadine transporter activity, particularly for high-throughput *in vitro* studies, a rapid, specific, and sensitive analytical method is needed. We have developed and validated an HPLC-MS/MS method for the quantification of fexofenadine in cell lysates from *in vitro* studies and in human plasma samples using certirizine as the internal standard. Following protein precipitation, analytes were separated by isocratic reverse-phase chromatography and analyzed by tandem mass spectrometry in the multiple reaction monitoring mode using the respective parent/daughter ions, *m/z* 502.5/466.2 for fexofenadine and *m/z* 389.0/200.9 for certirizine. The method exhibited a linear dynamic range of 0.5–500 ng/mL for fexofenadine in cell

lysates. The lower limit of quantification was 0.5 ng/mL with a relative standard deviation of less than 5%. Precision and accuracy were within the limits presented in the FDA guidelines for bioanalysis. Total analysis run time was 3 minutes per sample. This method has been successfully used to characterize the kinetics of fexofenadine transport by HeLa cells transiently-transfected with OATP1A2 and also has been applied to the determination of fexofenadine pharmacokinetics in human subjects. This validated method provides a rapid, sensitive, and specific analytical tool supporting the use of fexofenadine as a probe to study the contributions of individual membrane transporters in uptake and efflux of xenobiotics.

944 DETERMINANTS OF TRAFFICKING AND FUNCTIONAL ACTIVITY OF THE HETEROMERIC ORGANIC SOLUTE TRANSPORTER, OSTα-OSTβ

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The recently discovered heteromeric organic solute and steroid transporter (OSTα-OSTβ) appears to be a major basolateral transporter of bile acids and endogenous and exogenous steroids in a variety of tissues including kidney, intestine, adrenal gland, and liver. Recent studies show that the interaction of the OSTα and OSTβ is critical for membrane trafficking, post-translational modifications, and transport function; however, the individual roles of the two proteins in generating the functional transporter are unknown. The present study tested the hypothesis that the conserved cysteine-rich region in Ostα is involved in substrate binding and translocation, whereas the putative endoplasmic reticulum retrieval signal sequences RRK/RNR are involved in the trafficking of the two subunits. The seven cysteine residues of mouse Ostα were substituted with serine ($Ost\alpha_{C7/S7}$), and the RRK/RNR sequences were converted to alanine ($Ost\alpha_{RRK/AAA}$ and $Ost\beta_{RNR/AAA}$). Expression of wild type OSTα and OSTβ in HEK293 cells resulted in enhanced [^{3}H]taurocholate transport; however, only background transport activity was noted in cells co-transfected with $Ost\alpha_{C7/S7}$ and wild type Ostβ. Western blot analysis showed normal protein expression of $Ost\alpha_{C7/S7}$, and co-immunoprecipitation studies indicated that $Ost\alpha_{C7/S7}$ is able to interact with Ostβ. Moreover, bimolecular fluorescence complementation analysis confirmed that the $Ost\alpha_{C7/S7}$-Ostβ correctly localizes to the cell membrane, indicating that this mutation does not impair heterodimerization or trafficking, but leads to a loss of transport activity. In contrast, mutation of the putative RRK/RNR membrane sorting signal motifs had only small effects. In conclusion, the conserved cysteine-rich region of Ostα is not required for trafficking, but is required for transport function, consistent with the hypothesis that it is involved in substrate binding. In contrast, the RRK/RNR sequences are not sorting signals for this heteromeric transporter. (Supported by DK067214, ES01247, and ES07026).

945 ABSORPTION AND DISTRIBUTION OF ^{51}CHROMIUM(VI) AND ^{51}CHROMIUM(III) IN MALE RATS AND MICE.

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Chromium is an element that exists in several valence states. Chromium(VI) [Cr(VI)] is an established human lung carcinogen whereas chromium(III) [Cr(III)] is not. The US National Toxicology Program (NTP) has recently finished a 2-year toxicology and carcinogenicity study of Cr(VI) administered to F344 rats and B6C3F1 mice as $Na_2Cr_2O_7$●$2H_2O$. As a support to the NTP study, we have investigated the absorption and distribution of orally administered ^{51}Cr(VI) as $Na_2{}^{51}CrO_4$ and ^{51}Cr(III) as $^{51}CrCl_3$ in both fasted and fed male F344 rats (2 µmol/kg) and male B6C3F1 mice (4 µmol/kg). Oral dose formulations contained (1) $Na_2{}^{51}CrO_4$ and unlabeled $Na_2Cr_2O_7$●$2H_2O$ (2) $^{51}CrCl_3$ and unlabeled $CrCl_3$●$6H_2O$, and (3) $^{51}CrCl_3$ and unlabeled $Na_2Cr_2O_7$●$2H_2O$ in deionized water. Blood and GI tissues without contents (separated into forestomach, glandular stomach, duodenum, jejunum, ileum, cecum, and large intestine) were counted on a gamma counter 24 h after dosing. The recovery in the blood and GI tissues of mice dosed with ^{51}Cr(VI) is 5.1% (fasted) and 2.6% (fed); the difference is from the % dose in blood (3.9% vs. 2.2%) and in GI tissues (1.2% vs. 0.4%). In comparison, less than 0.5% of the ^{51}Cr(VI) dose was recovered in the blood and GI tissues of rats. Fasted rats had a higher % of the ^{51}Cr(VI) dose in the blood than fed rats (0.19% vs. 0.06%), but both fasted and fed rats have a similar % dose in GI tissues (~0.3%). In all ^{51}Cr(III) studies, less than 0.5% of the doses was recovered in the blood and GI tissues. The absorption of $^{51}CrCl_3$ and unlabeled $CrCl_3$ to the blood and GI tissues of mice (0.4% in both fasted and fed) is better than the absorption $^{51}CrCl_3$ and unlabeled $Na_2Cr_2O_7$ in mice (0.17% fasted and 0.04% fed), but the absorption of these two Cr(III) formulations are similar in fasted and fed rats (up to 0.05%). In conclusion, Cr(VI) is better absorbed than Cr(III) in both mice and rats. Mice absorb both Cr(VI) and Cr(III) better than rats. Food decreases the absorption of Cr(VI) in both mice and rats. [Supported by NIEHS Contract No. N01-ES-25483 and HHSN291200775562C]

946 TOXICITY AND TISSUE DISTRIBUTION OF TITANIUM DIOXIDE (TIO2) NANOPARTICLES IN SUBCUTANEOUSLY AND INTRAVENOUSLY INJECTED MICE OVER 6 MONTHS.

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Nanomaterials are becoming more prevalent in medical and consumer products yet there is a paucity of knowledge in understanding the toxicology of nanoparticles. The objective of this study was to evaluate the tissue distribution of TiO2 nanoparticles (20nm, Degussa) up to 6 months after exposure. Particles suspended in sterile saline were injected into Balb/c mice on days 1 and 2. Mice received either 4 subcutaneous (SC) injections in the inguinal and axillary areas, or 2 tail vein injections (IV). The total doses were 5600 or 560 mg/kg (SC); or 560 or 56 mg/kg (IV). Necropsies were performed at 2, 4, 12, and 26 weeks after dosing. No biologically significant changes were seen in major circulating leukocyte populations. In the SC injected mice, most of the injected particles remained at the injection site as a solid aggregate and a typical "foreign-body" response was generated. This deposit decreased over time and particle aggregates could be detected in macrophages entering the adjacent tissues and draining lymphoid tissues. Although there was considerable variation between animals, aggregates of TiO2 were typically seen in the tissues examined at all time points and doses except aggregates from low dose treatments were not visible in as many tissues and at as many time points. ICP-MS analysis showed no decrease in TiO2 in liver, lung, and spleen at 26 weeks. Aggregate formation may be an important feature of the response to TiO2 nanoparticles in tissues but there remains a background level of unaggregated nanoparticles that may also contribute to cellular responses. TiO2 nanoparticles appeared to remain in tissues where they initially localize, and were not cleared over a 6-month period. However, we do not have evidence of any toxic effects associated with these aggregates.

947 CHARACTERIZATION OF PRESYSTEMIC ELIMINATION OF TRICHLOROETHYLENE (TCE) IN RATS FOLLOWING ENVIRONMENTALLY-RELEVANT EXPOSURES.

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TCE is volatile organic chemical (VOC) that has been used extensively as a degreaser, solvent and dry cleaning agent. As a result of its widespread use, TCE is the most common contaminant in groundwater at U.S. hazardous waste sites. Human exposure to TCE is of concern, because high, chronic doses are carcinogenic in mice and rats. In order to characterize the toxicokinetics (TK) of TCE following ingestion of environmentally-relevant (i.e., trace) doses, a valid, sensitive and rapid analytical method was developed using headspace solid-phase microextraction (HS-SPME) and gas chromatography with negative chemical ionization (NCI) mass spectrometry (GC-NCIMS). The lower limit of quantitation was 0.025 ng/ml blood. The right carotid artery of male Sprague-Dawley rats was cannulated. TCE was given as an oral bolus or iv, in doses of 0.0001, 0.001, 0.01, 0.1, 1.0, 2.5 and 5.0 mg/kg in an aqueous Alkamuls® emulsion. Serial 200-µl blood samples from each rat were taken and analyzed immediately to obtain concentration-time profiles. WinNonlin software was used to determine TK parameters. TCE concentrations were measurable at the 0.001 mg/kg dose for only the first 15 min post dosing and not detectable at the 0.0001 mg/kg dose. At 0.01 mg/kg, TCE was detectable for 120 min. No terminal elimination phase was present. TK parameters could be ascertained for 0.1-5.0 mg/kg. Bioavailability ranged from 6 to 11 %, but was not dose-dependent from 0.1-5.0 mg/kg. Clearance ranged from 51.2 to 60.6 ml/min-kg and was constant in this dosage range. A minuscle amount of TCE (AUC015 = 0.9 ng-min/ml) reached the systemic circulation of rats ingesting 0.001 mg/kg. This dosage would be received by a 70 kg person who consumed 2 L of water containing 35 ppb TCE. Our analytical method makes it possible to directly assess the efficiency of presystemic elimination of trace levels of TCE. Supported in part by DOE Coop. DE-FC09-02CH111090)

948 INHALATION KINETICS OF JET FUEL COMPONENTS IN THE RAT.

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Jet Propellant-8 (JP8) is a complex mixture of aromatics and aliphatics. The pharmacokinetics and toxicology of several aromatic hydrocarbons found in JP-8 are well characterized, but hydrocarbons representing the heavier end of the fuel are less

studied. To better understand the inhalation kinetics of this fraction of the fuel two 4-hour aerosolized inhalation studies (1269 and 661 mg/m3) were carried out with rats exposed to a 1:1 mix by mass of six liquid n-alkane hydrocarbons (decane, undecane, dodecane, tridecane, tetradecane, pentadecane) and two crystalline aromatics (naphthalene, 2-methylnaphthalene) dissolved in the liquid hydrocarbon mixture. Nose-only chamber atmospheres contained aerosol droplets (66-70%) and the remainder vapor. Aerosol droplet composition ranged from 30% (decane) to 99% (pentadecane). Uptake of hydrocarbons in the lung, blood, brain, and liver was evaluated during exposure, and clearance of the chemicals was monitored for up to four hours post-exposure in these tissues. Alkane concentrations were greater in lung compared to other tissues, while naphthalene and 2-methylnaphthalene achieved greater post-exposure concentrations in liver and brain compared to the lung for most time points. Pentadecane and tetradecane cleared from the body more slowly than other hydrocarbons. Interestingly, after exposure ceased, pentadecane concentrations continued to increase in blood and liver for up to 45 min. Biological determinants controlling hydrocarbon clearances from the sampled tissues are hypothesized to be fat deposition and/or metabolic clearance. Concentrations of all hydrocarbons decreased 40-100% by 4 hours post-exposure. (AFOSR Grant #: AFOSR-2006-0010A)

949 THE EFFECT OF ANAESTHETICS AND DOSE FORMULATIONS ON THE PRODUCTION OF TOXICOKINETIC DATA.

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Toxicokinetic data provides quantitative information on exposure of animals to a test compound during a toxicity study. Measurements of the test compound are commonly carried out on samples obtained during the study using the technique of liquid chromatography in combination with mass spectrometry (LC-MS/MS).LC-MS/MS methods are susceptible to interference from components present in the sample matrix. These can cause suppression or enhancement of the measurement of the test compound ions in the mass spectrometer, known as a matrix effect. This is problematic if the matrix effect only influences the measurement of either the samples or the calibration standards and quality controls (QCs). Matrix effects may lead to large inaccuracies in study data.The presence of a barbiturate anaesthetic in control plasma used in a primate toxicity study and the formulation of the dose vehicle administered during a rodent study affected the measurement of plasma and blood concentrations respectively of two different test compounds using validated LC-MS/MS methods. In both examples, the methods were affected by ion suppression or enhancement caused by the matrices that were analysed. Calibration standards and QCs met the appropriate acceptance criteria. This led to a reduction (50%) in the first case and an increase (50%) in the second case in apparent measured concentrations of test compounds in samples, with a knock on effect to pharmacokinetic parameters. Matrix effects were identified using in-house software. The first case was solved by sourcing and screening alternative batches of primate plasma. The second case was resolved by diluting samples with control blood prior to analysis.In conclusion, careful observation is required when LC-MS/MS methods are implemented for routine sample analysis as batches can meet acceptance criteria for calibration standards and QCs, but other factors have to be considered before incurred sample data can be reported.

950 PHARMACOKINETIC MODEL OF CARISOPRODOL AND MEPROBAMATE METABOLISM IN HUMANS.

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Carisoprodol is one of the most widely prescribed muscle relaxants in the U.S. and is also a drug known to be a subject to abuse. The literature contains a number of cases where carisoprodol was involved in intentional overdoses or accidental intoxications. Despite the fact that carisoprodol has been available for prescription since 1959, a number of gaps in our knowledge of the toxicokinetics of this common drug exist. For example, neither the volume of distribution nor the whole blood-plasma partition coefficient for carisoprodol in humans have been reported. We have developed a two compartment pharmacokinetic model describing carisoprodol metabolism and that of the primary metabolite, meprobamate. The model accounts for first pass metabolism of carisoprodol and was able to replicate the data from several previously reported datasets (Kucharczyk et al., 1986, Olsen et al., 1994, and Bramness et al., 2005). Based on an analysis of the data, the volume of distribution for carisoprodol was found to be 1.1 L/kg while that for meprobamate was 0.8, consistent with values previously reported in the literature. The rate constants for absorption of carisoprodol, metabolism to meprobamate, and meprobamate elimination, were found to be 2.1, 0.7, and 0.13 per hour, respectively. Due to the substantial differences in the half-lives of carisoprodol and meprobamate, the model may have significant utility for estimating doses of carisoprodol in cases of suspected overdose.

951 PHARMACOKINETIC EVALUATION OF EXPOSURE TO ECOLOGICALLY RELEVANT CONCENTRATIONS OF 2, 3, 7, 8-TCDF AND 2, 3, 4, 7, 8-PECDF IN MINK (MUSTELA VISON).

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Wild mink (Mustela vison) living along the Tittabawassee River in central Michigan exhibit elevated hepatic and dietary polychlorinated dibenzofuran (PCDF) concentrations exceeding mink-specific, literature-reported Toxicity Reference Values (TRVs) on a Toxicity Equivalents (TEQ) basis. However, no apparent effects on individuals or population are evident, suggesting that available TRVs may over-predict risk for the site-specific mix of congeners. To investigate this discrepancy, a 180-day spiked feed study was conducted to assess: 1) the dosages of key congeners necessary to achieve liver concentrations bracketing those observed in wild mink; 2) time to achieve steady state concentrations; and 3) effect of co-administration of 2,3,7,8-tetrachlorodibenzofuran (TCDF) and 2,3,4,7,8-pentachlorodibenzofuran (4-PeCDF) on the pharmacokinetics and distribution of each congener. Adipose and hepatic PCDF concentrations were measured at 0, 90, and 180 d. PCDFs concentrations in mink scat were determined at several time points and indicated nearly complete absorption of both TCDF and 4-PeCDF from the diet. Elimination half-times of TCDF were <15 h and were inversely proportional to dose, while those for 4-PeCDF were approximately 7 to 9 d with no clear dose-dependency in the tested dose range. Co-administration of 4-PeCDF and TCDF accelerated clearance of TCDF compared to administration of TCDF alone. Clearance of 4-PeCDF was not affected by TCDF co-administration. Distribution of 4-PeCDF, but not TCDF, demonstrated increased hepatic sequestration with increasing dose. The results suggest that these two compounds are less persistent in wild mink than predicted on the basis of laboratory rodent data and will facilitate design of further site-specific chronic toxicity studies.

952 DOSE-DEPENDENT DISTRIBUTION AND ELIMINATION OF CIS- AND TRANS-PERMETHRIN IN THE RAT.

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Pyrethroids are neurotoxic insecticides used in a variety of agricultural and household activities. Due to the phase-out of organophosphate pesticides, use of pyrethroids has increased. The potential for increased human exposure to pyrethroids has prompted pharmacokinetic research to better characterize exposure. This work tested the hypothesis that the disposition of permethrin into blood, brain, liver and fat compartments in the rat is dose-dependent. Adult male Long Evans rats were orally gavaged with permethrin (40:60 cis:trans) in corn oil at 1 ml/kg. Dose-tissue concentration relationships were assessed by dosing rats with 1, 3, 10, 30 or 100 mg/kg and sacrificing at 2 hr (n=4 rats/dose). Tissues were removed, extracted and analyzed by HPLC/MS/MS for parent isomer. Increasing doses resulted in increasing tissue concentrations. At each dose, blood and brain concentrations were not different. Permethrin tissue concentrations over time in blood, brain, fat and liver were also assessed. Rats were dosed with 1 or 10 mg/kg and sacrificed at 1, 2, 3, 4, 6, 12, 24, 36 or 48 hr (n=4 rats/time point). Permethrin concentrations were greater in rats dosed with 10 mg/kg than 1 mg/kg in all tissues. Elimination patterns were similar for both isomers. However, area under the curve (AUC) was greater for cis- relative to trans-permethrin in all tissues. Relative rankings of AUC in each of the tissues were fat>blood>brain>liver. AUCs were 10-fold greater in the 10 mg/kg than 1 mg/kg rats for blood, fat and liver. However, trans-permethrin concentrations in the brain were not different between the dose levels ($p>0.05$). Permethrin distributes rapidly throughout the rat after oral administration. Over time, distribution of permethrin is dose-dependent, except for trans-permethrin in brain. Isomer specific distribution of permethrin may impact the neurotoxic effect of this pesticide. (This abstract does not represent US EPA policy).

953 ALTERATION OF PERMETHRIN PHARMACOKINETICS FOLLOWING A SINGLE DERMAL DOSE OF DEET (N, N-DIETHYL-M-TOLUAMIDE), ALONE OR IN COMBINATION WITH AN ORAL DOSE OF PYRIDOSTIGMINE BROMIDE (PB) IN MALE RATS.

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We studied effect of a dermal dose of the insect repellent, DEET (400 mg/kg), alone or with an oral dose of the nerve agent prophylaxes, PB (13 mg/kg) on the pharmacokinetic profile of a dermal dose of (1.3 mg/kg) the insecticide permethrin

in male rats. Blood, tissues, and urine samples were collected at 0.5, 1, 2, 4, 8, 24, 48 and 72 hr after dosing. Plasma, liver, kidneys, brain, testes, stomach and urine were analyzed. The application site of the skin retained approximately 0.5% of permethrin following administration, 72 hr after dosing, either alone or in combination with DEET, or PB. Permethrin and its metabolites m-phenoxybenzyl alcohol and m-phenoxy benzoic acid were identified in rat plasma, tissues and urine samples. Concurrent application of three compounds resulted in a systemic exposure to permethrin as the following: 65.6, 104.2, 108.7 and 131.2 µg.hr/mL, following application of permethrin, permethrin with DEET, permethrin with PB and permethrin with DEET and PB, respectively. The combined administration of DEET and PB with permethrin increased the half-life of elimination of permethrin from 10.5 to 17.8 hour and decreased the urinary excretion of permethrin metabolites compared to its application alone. These results demonstrate that an interaction was observed between permethrin, DEET and/or PB, with a more profound effect from PB, indicating possible competition for metabolism enzymes/distribution processes in the male rat. Alterations of the pharmacokinetic profiles in the presence of the other compounds may explain previous reports of increased toxicity following combined exposure of test compounds that may have contributed to the Gulf War Veterans illnesses. Supported by the USAMRMC DAMD 17-99-1-9020.

954 *IN VIVO* DERMAL ABSORPTION OF PYRETHROIDS IN THE RAT.

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The potential for exposure to pyrethroid pesticides has risen recently because of their increased agricultural and residential use. The objective of this study was to examine the in vivo dermal absorption of bifenthrin, deltamethrin and cis-permethrin in the rat. Hair on the dorsal side of adult male Long-Evans rats was clipped with an electric razor. The next day, the rats were anesthetized and dosed with 1750 nmole (312.5 nmole/cm2) of radiolabeled (5 uCi) bifenthrin (B), deltamethrin (D) or cis-permethrin (P) in acetone. A non-occluding plastic cover was glued to the skin over the dosing site. The animals were placed in individual metabolism cages for the collection of urine and feces. The next day, the animals were anesthetized and the dosing site was washed soap and water. Then the animals were sacrificed by cardiac puncture and several tissues were collected. The washed skin was also tape stripped. The excreta, skin wash, tape strips and tissues were analyzed for radioactivity. The wash removed the greatest percentage of the dose: B, 52.3 ± 4.3% (mean ± SD, N=4); D, 62.6 ± 3.1 %; P, 62.3 ± 3.7%. Of the tissues, the skin had the greatest percentage of the dose: B, 24.1 ± 5.0%; D, 20.1 ± 2.4%; P, 18.7 ± 3.3%. The percentage of the dose removed by tape from the skin was: B, 5.2 ± 1.8%; D, 3.6 ± 0.6%; P, 5.1 ± 3.6%. In urine, the percentage of the dose detected was: B, 0.6 ± 0.1%; D, 0.2 ± 0.02%; P, 3.5 ± 0.9%. In feces, the percentage of the dose detected was: B, 2.0 ± 0.7%; D, 0.3 ± 0.2%; P, 3.7 ± 1.6%. Less than 0.01% of the dose for each of the pyrethroids was detected in the internal tissues analyzed. Complete absorption of the pyrethroids tested through rat skin was low (1-7%). However, a considerable amount of these pyrethroids remained in skin (19-24%) and could potentially be available for systemic absorption. (This abstract does not represent US EPA policy.)

955 ONTOGENY OF METABOLISM OF VOLATILE ORGANIC COMPOUNDS (VOCS) IN SPRAGUE-DAWLEY (S-D) RATS.

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Cytochrome P450 (CYP) enzymes play an important role in the metabolism and disposition of a wide range of xenobiotics. Published experimental findings indicate that CYPs and conjugative enzymes mature at different rates in the liver of human infants. Relatively little information is available on the maturation of hepatic CYPs in rats, a commonly used animal model. Altered expression of CYPs may substantially modify toxic/carcinogenic effects of VOCs in young test animals. Previous data from our laboratory demonstrated age-related changes in CYP2E1, CYP2B1/2 and CYP1A1/2 activities in unsexed S-D rats. In each case, enzymatic activity peaked on postnatal day (PND) 21, followed by a decrease in activity until adulthood. The objective of this study was to characterize the ontogeny of hepatic metabolism of dichloromethane (DCM), trichloroethylene (TCE) and perchloroethylene (PERC). CYP2E1 and CYP2B1/2 are primarily responsible for the metabolism of low and high doses, respectively, of each VOC. In our study we determined the Michaelis-Menten metabolic parameters, Km and Vmax, to establish VOC metabolic capacity for PNDs 10, 15, 21, 60 and 90. Our data reveal a peak in Vmax for each chemical at PND 21, with relatively little change in Km during this maturation period. DCM and TCE showed an increase in metabolism with increase in substrate concentration, while there was relatively little metabolism of PERC on PNDs 10 and 15. These age-dependent metabolic rate constants will be incorporated into physiological models for use in predicting the disposition of DCM, TCE and PERC in immature test animals.

956 SODIUM PERFLUOROHEXANOATE PHARMACOKINETICS IN RATS DURING AND AFTER 90-DAY ORAL GAVAGE ADMINISTRATION.

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The pharmacokinetics (PK) of sodium perfluorohexanoate (PFHxNa) were evaluated in rats during & after a 90-day oral dose toxicity study. Four groups, 10 rats/sex/group, were administered 0, 20, 100 or 500 mg/kg/day by gavage. Blood was collected at three 24-h periods during dose administration on Days 0, 28, & the last day of dosing (92 males/93 females), from 2-3 rats/sex at 0.5, 1, 2, 6, 12, & 24 h after administration of the daily dose. Blood samples also were collected during the recovery phase from the 500 mg/kg/day dose group only, on Days 93/94 to 99/100, 104/105, 112/113, 118/119 & 183/184, to estimate the terminal elimination half-life ($T^{1/2}$). Tissue samples (blood, fat, liver, & kidney) from the high dose (10/sex/group) were collected during the recovery phase to assess changes in tissue:plasma ratios on Days 92/93, 120/121, & 183/184 for male/female rats, respectively. Plasma and tissue samples were extracted and analyzed by LC/MS/MS. PK parameters (Cmax, $T^{1/2}$, AUCINF) were calculated. The 24-h plasma concentration data indicate that PFHxNa was readily absorbed. Tmax ranged from 0.5 to 1 h. Cmax & Cmax/dose values were generally similar at the three collection times during dose administration. A reduction in internal dose (AUCINF) was observed at the highest dose in male but not female rats. The terminal $T^{1/2}$ was 14.9 & 7.9 days in males and females, indicating slower excretion by male rats. The portion of the internal dose remaining in plasma 24 h after the last dose was 24% & 7% of the total AUCINF calculated from Day 92/93 through the end of the recovery phase for male and female rats, respectively. Final plasma concentrations were less than 0.008% of the Day 92/93 Cmax values. Tissue concentrations continued to decline in both sexes during the recovery phase. Tissue:plasma ratios indicate slightly greater retention in fat & kidney compared with liver. These results can be used to aid interpretation of toxicity endpoints and provide a basis for comparison with pharmacokinetic data from other studies.

957 8-2 FLUOROTELOMER ALCOHOL: LIVER GLUTATHIONE STATUS, METABOLITE KINETICS IN TISSUES, AND EXCRETION AND METABOLISM WITH DAILY ORAL DOSING.

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Rats were administered 8-2 Fluorotelomer Alcohol (8-2 FTOH) by oral gavage for 45 days and livers were collected periodically to evaluate glutathione status; liver and selected tissues were analyzed for 8-2 FTOH and perfluorinated and polyfluorinated metabolites. On day 46, conditioned and naïve rats were administered [14C]8-2 FTOH and sacrificed 2 hours post dose to determine the percent of covalently bound radioactivity in liver, kidney and plasma. Also, conditioned rats were administered [14C]8-2 FTOH, maintained for 7 days, and a complete material balance performed along with metabolite identification. Lastly, selected samples were processed with 2,4-dinitrophenyl hydrazine (DNPH) and screened by LC/MS for aldehydes and ketones. Overall, liver glutathione, both oxidized and reduced, was unaffected by daily administration of 8-2 FTOH. The most ubiquitous metabolites in tissues were 7-3 Acid and Perfluorooctanoic Acid (PFOA); 8-2 FTOH had the greatest concentration in fat and most metabolites exhibited steady-state by day 25. There were no differences in the percent of covalently bound radioactivity in naïve and conditioned rats. Following dosing with [14C]8-2 FTOH, >74% was eliminated in the feces, which contained primarily 8-2 FTOH. Urine was a minor elimination pathway for PFOA, 7-2 secondary FTOH-glucuronide and 8-2 unsaturated FTOH N-acetyl cysteine (females only). The greatest percentage of the administered dose was found in the liver, fat and skin; in liver, the principle metabolites were PFOA, Perfluorononanoic Acid (PFNA), 8-2 FTOH-sulfate and 7-3 Acid. In the kidney, PFOA and 8-2 FTOH-sulfate was observed, and in plasma, 7-3 Acid, PFOA and 8-2 FTOH-sulfate was detected. DNPH-derivatized samples confirmed the presence of 7-2 Ketone, 7-3 Aldehyde and 7-3 α-β Unsaturated Aldehyde. These results provide a comprehensive evaluation of 8-2 FTOH pharmacokinetics and metabolism and may provide insight into the potential mechanisms of toxicity observed in the rat.

958 EVALUATION OF BIOPERSISTENCE POTENTIAL AMONG CLASSES OF POLYFLUORINATED CHEMICALS USING A MAMMALIAN SCREENING METHOD.

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A biopersistence screening method was used to evaluate the uptake and clearance of several classes of polyfluorinated chemicals in male rat blood. Each chemical was administered by oral gavage to groups of 5 rats each for 10 days at doses expected to be minimally toxic. Blood was collected 2 hrs after the first dose, and on days 5 and 10 of treatment and on days 13, 24, 52, and 94 post-treatment. The total organic fluorine content (ppm F) in blood was determined using a Wickbold torch combustion/fluoride ion electrode analysis method. Non-compartmental analysis was conducted on blood fluorine data. All data were normalized to µM equivalents and 0.1 mmol/kg body weight, based on molecular weight, dosage, percentage of active ingredient, and percent fluorine. The dose-normalized area under the blood-concentration curve (AUC/D) was calculated for each chemical and used as a comparative metric. The blood AUC/D for the perfluorinated carboxylic acids sodium perfluorohexanoate (PFHxNa), ammonium perfluorooctanoate (APFO), and perfluorodecanoic acid were 9068, 81507, and 482871, respectively. The blood AUC/D for the fluorotelomer alcohols (FTOHs) 2-perfluorohexylethanol (6-2 FTOH), 2-perfluorooctylethanol (8-2 FTOH), and 2-perfluorodecylethanol (10-2 FTOH) were 1066, 3757, and 1626 respectively. A FTOH mixture comprised of 50% 8-2 FTOH and 50% 10-2 FTOH and higher was evaluated resulting in blood AUC/D of 6242. Finally, fluorotelomer sulfonates (FTSs) were compared to perfluorooctane sulfonate (PFOS) and AUC/D values ranging from 17865 to 566500 were determined. PFOS had the highest AUC/D, 566500, and was the most biopersistent substance studied. These data indicate that perfluorinated acids, polyfluorinated fluorotelomer alcohols, and fluorotelomer sulfonates are very different from one another with regard to bioavailability and biopersistence in this assay.

959 EFFECT OF LOW TO MODERATE DOSES OF ETHYL ALCOHOL ON REACTION TIMES.

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It has been well established in the scientific literature that blood alcohol levels (BAL) of 80mg/dl or greater causes a significant prolongation in reaction times. Studies conducted in other laboratories have shown impairment on reaction times of complex tasks such as driving at doses less that 80mg/dl. In order to determine precisely the effect of lower BALs on reaction time, studies were conducted using volunteers who consumed alcoholic beverages at a dose of 0.6g/kg. The BALs for the volunteers were determined throughout the experiment by utilizing a breathalyzer (AlcoSensor IV). The volunteers then completed various simple (2 stimuli) and complex (5 stimuli) tasks on a driving simulator utilizing reaction time software (Vericom). At a BAL of 61mg/dl, complex reaction time increased by 30% ($p<0.05$) while for simple reaction times no significant increase was found. These results demonstrate that while individuals may have no significant deficit in simple tasks, complex tasks such as driving may be impaired and decrease the driver's ability to safely operate a motor vehicle.

960 DISPOSITION OF DICLOFENAC AND ITS METABOLITES IN WILD-TYPE AND *MRP3-NULL* MICE.

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Although the non-steroidal anti-inflammatory drug diclofenac is normally well tolerated, toxicities occur primarily from its metabolites. In particular, diclofenac-1-O-acyl glucuronide (DG) covalently binds to hepatic and intestinal proteins. The consequences of such adduct formation are altered protein function or possible immunogenic response. Individuals with polymorphic forms of Mrp3 may have altered susceptibility to adduct formation stemming from a potential increase in liver and intestinal xenobiotic levels. As glucuronides are substrates for the basolateral transporter Mrp3, we investigated the role of Mrp3 in DG hepatobiliary disposition. In the present study, an i.v. dose of diclofenac (3 mg/kg) was given to FVB wild-type (WT) and Mrp3-null (KO) mice. Bile and plasma were collected over a 90-min period, and at the end of the experiment livers were harvested. Diclofenac and its metabolites were quantified by LC/MS/MS. In the bile of both genotypes four different diclofenac metabolites were identified, the parent compound, DG, hydroxydiclofenac (OH-D) and an unknown metabolite (UD), possibly a glucuronide conjugate different from DG. In both genotypes the biliary excretion of DG was 10-fold higher than for the other metabolites. No differences in biliary, DG, and OH-D excretion rates were seen between WTs and KOs, but excretion of the UD metabolite was 2-fold greater in the KOs. During the 90-min experiment, the average plasma level of DG was over 80% lower in KO mice compared to WT mice. In the liver, diclofenac and OH-D were the dominant metabolites. There were no significant differences in the hepatic concentrations of all diclofenac metabolites. Further studies are in progress to identify UD and elucidate the toxicological significance of disparities in DG disposition between genotypes and the increased biliary excretion of UD in KO mice. Supported by NIH Grants DK069557 (JEM) and ES009716 (CDK).

961 STEREOSELECTIVITY IN DISPOSITION OF BROMOCHLOROACETIC ACID (BCA) IN FISCHER 344 RATS AND B6C3F1 MICE.

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The time courses of (+) and (-) isomers of bromochloroacetic acid (BCA) were evaluated following a single intravenous administration of BCA racemic mixture to male and female Fischer 344 rats (10 mg/kg) and B6C3F1 mice (100 mg/kg). Blood samples were collected at pre-determined time points and analyzed for plasma concentrations of BCA isomers using a validated gas chromatography method with electron capture detection (GC/ECD). Plasma concentration time data for (+)-BCA isomer in rats were characterized by a two-compartment model, whereas the other data sets were described by a non-compartmental analysis. (-)-BCA isomer exhibited much faster elimination from the systemic circulation than the (+)-BCA. The elimination half-life values were 4.46 and 5.52 minutes in the male and female rats for (+)-BCA isomer, whereas these values were 2.94 and 2.21 minutes for the (-)-BCA. For the mice, (+)-BCA isomer exhibited elimination half-life values of 4.88 and 4.86 minutes in males and females, while these values for the (-)-BCA were 1.92 and 1.80 minutes. The systemic exposures to BCA in rats and mice, as evidenced by AUC values, were approximately two times greater for the (+)-isomer than the (-)-isomer, which in turn contributed to smaller clearance of (+)-BCA than (-)-BCA. For the rats, the clearance values were 71.9 and 92.9 mL/min/kg in the males and females for (+)-BCA whereas these values were 126 and 206 mL/min/kg for (-)-BCA. For the mice, the clearance values were 115 and 98.9 mL/min/kg in the males and females for (+)-BCA whereas these values were 189 and 175 mL/min/kg for (-)-BCA. These results indicate a stereoselective disposition of BCA enantiomeric mixture, which potentially implicates a differential metabolism between two isomers of BCA. This study was supported by NIH (N01-ES-5456).

962 SINGLE ADMINISTRATION GAVAGE TOXICOKINETIC STUDY OF 2 METHYLTETRAHYDROFURAN (MTHF) IN FISCHER 344/N RATS AND B6C3F1 MICE.

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A TK study of MTHF, a component of alternative fuels (P-series fuels), was conducted in Fischer 344/N rats and B6C3F1 mice to correlate toxic effects with systemic availability and to improve the usefulness of toxicity study results in risk assessment. Rats and mice (12 to 13 weeks old) were given a single gavage administration of MTHF in Milli-Q water at dosages of 10, 30, 100, or 400 mg/kg for rats and 20, 100, or 400 mg/kg for mice. Blood samples were collected and analyzed for MTHF using a validated headspace capillary GC/MSD method for the low concentration range and a validated headspace capillary GC/FID method for the high concentration range. One-compartment models (first order or equal absorption and elimination) were used for the rat 10 and 30 mg/kg profiles and mice 20, 100, and 400 mg/kg [M only) profiles, whereas one- or two- compartment Michaelis-Menten (MM) models were used for the rat 100 and 400 mg/kg and mouse 400 mg/kg (F only) profiles. Absorption into the systemic circulation was very rapid. MTHF was measured in plasma within minutes after dosing even at the lowest doses tested. Absorption rate (0.1 to 0.8 min-1) increased with increasing dose for rats but not over the dosage range tested for the mice (~0.3 min-1). Elimination was very rapid and saturable. Half-life values were ~20 and 37 min at 10 and 30 mg/kg (rat) and ~4, 6, and 21 min at 20, 100, and 400 [M only] mg/kg, respectively. Vmax values were ~ 0.2, 0.3, and 3 ug/min and Km values ~7, 11, and 49 ug/mL for the 100 and 400 mg/kg rat and 400 mg/kg mice (F only) groups, respectively. A larger than proportional increase in AUC occurred with increasing dose at all dose levels for both species. This study shows that MTHF is readily absorbed into the systemic circulation following oral exposure; enhanced absorption and saturated elimination occurs in a dose- and species-dependent manner. This work was supported by NTP Contract N01-ES-05456.

963 APPROACHES FOR SETTING AN ANALYTICAL ASSAY LLOQ IN PRE-CLINICAL TOXICOKINETIC STUDIES.

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Generic methods of setting the bioanalytical assay Lower Limit of Quantitation (LLOQ) for toxicokinetic (TK) studies were assessed for impact on TK parameters. The LLOQ was set to either 10ng/mL or 5% of the Cmax from the low dose group on the first study. Using concentration data from previous studies, the lowest dose group data were modified such that concentrations below the new LLOQ were set to 0 and the AUC re-calculated. These results were reviewed to determine impact on AUC (change >15% of original), number of below the limit of quantitation (BLQ) samples and the number of instances of quantifiable concentration in controls (QCC). Using either approach the incidence of QCC decreased substantially. In most studies, changing the LLOQ resulted in loss of some or all of the low dose data points and AUC was impacted in the low dose group. Using an LLOQ of 10 ng/mL, AUCs were comparable to the original (67%), but trended towards a lower or no reportable AUC (33%). Using an LLOQ of 5% Cmax of the initial study was inadequate if subsequent studies were conducted at lower doses. In this case, 24% of the re-calculated AUCs were impacted, 88% of which were GLP studies. Using either approach, when the AUC changed, the AUCs were on average approximately ½ of their original values. It was also noted that in 69% studies evaluated, concentration data from other groups was also affected. In conclusion, modern LC-MS/MS technology allows increasingly sensitive assays. However, lower LLOQs incur the risk of incidents of carryover, selectivity issues and QCC that are not study impacting. Choice of an adequate LLOQ to ensure TK data for the NOAEL is critical. This modeling has shown that use of the generic LLOQ-setting method produced AUC results that were comparable to the original TK data in 67% or 76% of the studies when the LLOQ was set to 10 ng/mL or 5% of Cmax, respectively. In addition, both approaches increased the incidence of BLQ samples in all dose groups, but substantially decreased the incidence of quantifiable concentrations in control samples.

964 ABSORPTION, DISPOSITION, AND ELIMINATION OF 1-BUTYL-3-METHYLPYRROLIDINIUM CHLORIDE (BMPY-CL) FOLLOWING IV, ORAL OR DERMAL ADMINISTRATION IN MALE F-344 RATS.

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Studies were conducted to characterize the effects of dose and route of administration on the dispositional fate of BmPy-Cl in male Fischer-344 rats. Following IV administration of [14C]-BmPy-Cl (5 mg/kg), radioactivity detected in the blood decreased rapidly. Urinary elimination accounted for 86±12% of dose with 9% of the dose was recovered in the feces. The percent of dose eliminated in feces at 72 h following oral administration (50, 5, or 0.5 mg/kg) of [14C]-BmPy-Cl was 51±6, 68±4 and 65±7%, respectively. Serial daily oral administrations (5 days) of [14C]-BmPy-Cl did not alter the patterns of disposition or elimination. Following each administration, 88-94% of the dose was eliminated in a 24 h period, with 63-76% of dose recovered in the feces. Systemic bioavailability was determined to be 43%, a value similar to the % of dose recovered in urine following oral administration (29-38%). As assessed by dose recovered in urine and feces, [14C]-BmPy-Cl was moderately but variably absorbed when applied to the skin in different vehicles (6-23%). Urine was the major route of elimination following dermal administration. Radioactivity detected in urine was due to [14C]-BmPy-Cl, following all doses and routes of administration. BmPy-Cl appears to be moderately absorbed, and that which is absorbed is extracted by the kidney and eliminated in the urine as parent compound, independent of dose, number of doses, or route of administration. This research was supported by the NIEHS NTP Grant No. N01-ES-45529 and NIEHS-sponsored Southwest Environmental Health Science Center Grant Number P30-ES-06694.

965 BIODISTRIBUTION OF ^{3}H-PIGMENT YELLOW 74, A TATTOO INK PIGMENT, FOLLOWING INTRADERMAL ADMINISTRATION IN MICE TO MIMIC TATTOOING.

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Tattooing has become one of the more popular methods of personal artistic expression. The application of permanent makeup to the lips, eyes and eyebrows has also grown in recent years. A poll in 2006 estimated 24% of the US population had at least one tattoo. The few studies on the chemical composition have indicated that a variety of inorganic and organic pigments are used in tattoo and permanent makeup inks. We have shown that tattooing induces a wound similar to the tissue-repair kinetics as any other dermal wounding. We have also shown that some tattoo organic pigments (1) photodecompose in simulated solar light, (2) can be metabolized *in vitro*, and (3) alter gene expression in the skin and lymph nodes following tattooing. What is not understood is the biodistribution or pharmacokinetics of tattoo pigments following tattooing. In order to address this data-gap, Pigment Yellow 74 (PY74; CAS 6358-31-2) was tritiated using an iodination/^{3}H exchange reaction, and the ^{3}H-PY74 intradermally injected into female female SKH-1 hairless mice (5 µL injection, 39 nmol, 256 Ci/mol). The mice were housed 3 per cage in metabolism cages, and sacrificed at 2, 4, 8, 12, 24, 48, 96, 168, and 240 hours. The ^{3}H levels in all tissues and excreta were quantified following homogenization, solubilization, and scintillation counting. ^{3}H-PY74 distributed from the injection site to the liver and was excreted in the intestinal contents via the bile. Approximately 78% of the ^{3}H-PY74 was excreted into the feces with the majority excreted within the first 24 hours, while less than 5% of the dose was excreted into the urine. Significant levels of ^{3}H-PY74 were detected in the regional draining lymph nodes and liver. Pharmacokinetic analysis of the data is pending HPLC quantification of ^{3}H-PY74 and metabolites. These results are the first to examine the biodistribution of a tattoo ink pigment following intradermal injection, and suggest that significant distribution of the pigment to the liver occurs following administration.

966 USE OF A PHYSIOLOGICALLY-BASED PHARMACOKINETICS (PBPK) MODEL TO PREDICT INTERNAL DOSIMETRY OF ZINC PYRITHIONE (ZPT) IN RATS AND HUMANS.

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ZPT is an established, efficacious ingredient used in shampoos for the treatment of dandruff. Repeated, high-dose administration of ZPT to rats produces hindlimb muscle weakness, yet no such toxicity has been reported in humans despite more that 50+ years of exposure. To further understand relationships between toxicity and internal dosimetry of ZPT, a PBPK model was developed. The model simulates the kinetics of absorption, distribution, and excretion of pyrithione (PT) and its metabolites 2-(methylsufonyl)pyridine (2-MSP) and the S-glucuronide (SG) conjugates of 2-pyridinethiol and 2-pyridinethiol-1-oxide following single or repeated oral administration of ZPT to rats. The model was initially parameterized and optimized based on data from single-dose intravenous and oral pharmacokinetics studies of pyrithione carbon (PTC, 14C from administered [14C-pyridine]-ZPT) in the rat; and evaluated and refined based on data from a repeated, multi-dose oral study of 14C-ZPT in the rat. The model replicated the observed short-term elimination kinetics of PT and PTC following single doses and longer-term temporal patterns of blood concentrations during repeated dosing schedules. The model also accounted for the production and rapid elimination of SG in urine, as well as production and slower elimination of 2-MSP, the major PTC species in blood within several hours following a dose of ZPT. The rat model was allometrically scaled to humans and used to calculate human and rat oral doses of ZPT that corresponded to the same plasma area under the curve (AUC) for PT. The human external dose of ZPT equivalent to the oral NOEL in the rat (500 µg/kg/day) was 2260 µg/kg/day. In human clinical studies, systemic exposure to ZPT has been estimated to be <5 µg/kg/day based on urinary excretion of PTC following topical application of product containing therapeutic concentrations of ZPT for 4 consecutive days. The PBPK model provides supportive evidence of the human safety of ZPT used in antidandruff shampoos.

967 PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL DEVELOPMENT AND SIMULATIONS FOR ETHYLENE DICHLORIDE (1, 2-DICHLOROETHANE) IN RATS.

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Under an Enforceable Consent Agreement (ECA) for 1,2-dichloroethane (ethylene dichloride, EDC, CAS No. 107-06-2), the U.S. Environmental Protection Agency has agreed to consider the use of physiologically based pharmacokinetic (PBPK) modeling for route-to-route extrapolation of existing and future toxicity studies conducted by the oral route for quantitative evaluation of potential hazards posed by inhalation of EDC. PBPK models for the disposition of EDC by rats have been previously described, but a need to update the model structure and parameter values was identified. The updated model reflects the current understanding of kinetics of conjugation reactions mediated by glutathione-S-transferases, the kinetics of glutathione turnover, availability of published reviews of physiological values commonly used in PBPK models, and consideration of kinetic data that were not part of the development of previous models. The available kinetic database includes studies in four strains of rats, single and repeated exposures, open chamber and closed chamber inhalation, and gavage studies using aqueous and corn oil dosing vehicles. The data collected in these studies included EDC concentrations in cham-

ber air, exhaled breath, blood, tissues, and gastrointestinal tract contents; liver, lung, and kidney GSH concentrations; and metabolite levels in urine. The updated model structure was used to recalibrate chemical-specific disposition parameters. The relative abundance of kinetic data for EDC in rats poses additional complexity in the evaluation of model fit. Despite some discrepancies in fit for the inhalation modeling, the PBPK model herein should be considered adequate for the purpose of conducting route-to-route extrapolations (oral to inhalation) for acute and repeated-exposure toxicity studies of EDC in male and female rats of various strains, on the basis of internal dose metrics such as blood and tissue EDC concentrations and production of GSH metabolites.
Funded by the HAP Task Force, a testing consortium of US EDC producers

968 ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION OF INTRAVENOUSLY AND ORALLY ADMINISTERED BIS-2-(BROMOMETHYL)PROPANE-1, 3-DIOL IN MALE FISCHER-344 RATS.

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Bis-2-(bromomethyl)propane-1,3-diol (BMP) is a brominated flame retardant that has been reported by the National Toxicology Program to be a multi-site carcinogen in rats and mice. To characterize the toxicokinetics, disposition and elimination of [^{14}C]-BMP, it was administered to male F-344 rats. Following a single IV administration of [^{14}C]-BMP (10 mg/kg) to jugular vein-cannulated rats, the radioactivity detected in whole blood disappeared rapidly. HPLC-radiometric analysis revealed only parent compound in blood. The half-life of elimination ($t_{1/2}$) and systemic clearance of [^{14}C]-BMP from blood (CL_b) were 5.2 min and 2.5 mL/min, respectively. After IV administration greater than 80% of the administered radioactivity was recovered from the urine by 12 h. Similar elimination profiles were obtained following a single oral administration (100 or 10 mg/kg), 92-100% of the dose of [^{14}C]-BMP was excreted in the urine within 72 h. Fecal elimination accounted for 7-10% of the dose. [^{14}C]-BMP radioactivity was recovered in the urine as a monoglucuronide conjugate. Less than 1% of a 100 mg/kg oral dose was retained in tissues at 72 h. These results indicate that BMP was rapidly absorbed in the gastrointestinal tract, efficiently metabolized to a glucuronide conjugate in the liver, and excreted in the urine. This research was supported in part by the NIEHS NTP Grant No. N01-ES-45529 and NIEHD-sponsored Southwest Environmental Science Center Grant No. P3-ES-06694.

969 STUDY OF THE AGGREGATE EFFECTS OF INGESTION, INHALATION AND DERMAL EXPOSURE TO THE INTERNAL DOSE OF TOLUENE IN RATS.

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The contribution of dermal and inhalation routes of exposure to volatile organic chemicals (VOCs) in drinking water is increasingly drawing attention. In the absence of human or field data, there are no reliable approaches available to evaluate the relevance and magnitude of these routes of exposure to VOCs in drinking water. The general objective of this study was to develop an experimental animal model to evaluate the contribution of multi-route exposures to the total dose of drinking water contaminants. Towards this goal, this study characterized the kinetics of toluene (TOL), a VOC found in drinking water, via various routes of exposure. Groups of 5 male Sprague-Dawley rats were given a single low or high dose of TOL by oral ingestion (ING: 8.3 or 32.5 mg/kg), inhalation (INH: 50 or 200 ppm × 2 hr) or dermal route (DER: 82 or 326 mg/kg). Two other groups were exposed to TOL by all three routes at the low doses or high doses given simultaneously. Blood samples (between 25 µl and 200 µl) were collected up to 6 hr after treatments and TOL concentrations were measured using a gas chromatograph equipped with FID. The blood concentrations (mean ± standard deviation) measured after multiroute exposure to the high dose of TOL were 8.3 ± 0.3 mg/L and 2.0 ± 0.7 mg/L, at 2 hr and 4.5 hr post-exposure. These levels were higher (by about 150 and 300 %) than the sum of the blood levels obtained after administration of TOL by each route: 5.5 and 0.7 mg/L after 2 hr and 4.5 hr. Compared to single route exposures (ING = 0.85 hr-1 ; INH = 0.83 hr-1 ; DER = 0.64 hr-1) multiroute exposure involving the high dose of TOL was associated with the lowest elimination rate constant (ING = 0.59 hr-1). These results suggest that the internal dose of TOL following multiroute exposures can not be always predicted by assuming additivity of internal dose associated with individual routes, and this is likely to be explained by a certain degree of saturation of metabolism during aggregate exposures.(Supported by NSERC, Canada).

970 PHARMACOKINETICS OF BIS-METHYLSALICYLATE CARBONATE (BMSC) AND ITS HYDROLYSIS TO METHYLSALICYLATE (MS) AND SALICYLIC ACID (SA) AFTER A SINGLE ORAL (GAVAGE) DOSE TO RATS.

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BMSC is an industrial chemical that is a dimer of MS. In aqueous solution, BMSC has been shown to hydrolyze to MS and ultimately SA. Developmental toxicity, including teratogenicity, in rats, typical of SA, was observed following oral BMSC dosing. SA is a well known rodent, but not human, developmental toxicant. Confirmation of the hydrolysis of BMSC following gavage administration was therefore required to confirm the proposed mechanism of SA-induced teratogenicity. Two groups of 16 female rats received a single 1000 mg/kg dose of BMSC or MS. Plasma was collected prior to dosing and at 10, 15, 30, 45 minutes and 1, 1.5, 3, 4.5, 6, 9, 12 and 24 hours post dosing. Urine was collected over 24 hours post-dosing. Plasma and urine samples were analyzed for BMSC, MS and SA by HPLC with UV detection. Results following BMSC dosing: MS and SA, but not BMSC, were identified in plasma and urine; MS and SA were detectable in plasma from 10 min to 24 h post-dosing; plasma MS peaked at 15 min post-dosing (4.14 µg/mL) whereas plasma SA peaked at 4.5 h post-dosing (285 µg/mL) and remained relatively constant; internal exposure to MS (AUC0-24 = 8.34 µg×h/mL) was 700-fold lower than internal exposure to SA (5,836 µg×h/mL); ~ 1% and 10% of BMSC was excreted in the urine as MS and SA, respectively. Results following MS dosing: MS in plasma peaked by 10 min post-dosing (15.6 µg/mL) then rapidly decreased (< 1 µg/mL at 1 hr) and plasma SA peaked at 30 min post-dosing then remained relatively constant (526 µg/mL at 24 h); the internal MS exposure (AUC0-24 = 3.82 µg×h/mL) was >2,500-fold lower than exposure to SA (10,163 µg×h/mL); < 0.5% and ~15% of MS was excreted in urine as MS and SA, respectively. These results confirm that no internal exposure to parent BMSC occurs following oral dosing and therefore, the developmental effects observed previously resulted from its hydrolysis products, MS and SA.

971 SINGLE INTRAVENOUS ADMINISTRATION TOXICOKINETIC STUDY (TKS) OF GLYOXYLIC ACID MONOHYDRATE (GXA) USING MALE AND FEMALE FISCHER 344 RATS.

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GXA is a metabolite of dihaloacetic acids, which are of toxicological importance as byproducts of chlorine-disinfected drinking water. A toxicokinetic (TK) study of GXA was conducted in Fischer 344/N rats to correlate toxic effects with systemic availability and to improve the usefulness of toxicity study results in risk assessment. Rats (11 to 15 weeks old) were given a single intravenous (IV) administration of GXA in normal saline at a dosage of 50 mg/kg (2 mL/kg). Blood samples were collected and analyzed for plasma GXA concentrations using a validated liquid chromatography method with mass spectrometry (LC/MS/MS) following protein precipitation (LOQ was 1.980 µg/mL). The GXA plasma concentration time profiles were biphasic and a two-compartment model with first order elimination provided the best-fit curves. The observed C2 min was 91 and 74 ug/mL (M & F). For males and females, the alpha, beta, and overall elimination half-life values were ~2, 13, and 3 min, respectively; clearance and MRT were ~80 mL/min/kg and 10 min, respectively; and Vss was ~760 mL/kg. AUCinf was 648 and 612 hr.ug/mL (M & F). There were no apparent gender-related differences in the GXA TK parameters. These results indicate that GXA is widely distributed and rapidly eliminated following IV administration in F344 rats. [This study was supported by NIEHS Contract No. N01-ES-55551]

972 EFFECT OF DRINKING WATER PRE-CONDITIONING ON TOXICOKINETICS OF BROMOCHLOROACETIC ACID (BCA) IN FISCHER 344 RATS AND B6C3F1 MICE.

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Bromochloroacetic acid (BCA) has been detected in drinking water as a result of chlorine disinfection. Due to the potential for toxicity, NIEHS selected BCA for toxicity testing and, in support of these studies, toxicokinetic (TK) studies were conducted to evaluate whether repeated exposure via the drinking water (pre-conditioning) affects the kinetics of BCA. Male and female F344 rats and B6C3F1 mice were used. A single gavage administration at a dosage of 10, 40, or 100 mg/kg was given to the rats, and 100, 200, or 400 mg/kg to the mice. Pre-conditioned animals were given BCA in the drinking water for 14 days (ad libitum), untreated drinking

water (overnight on Day 14), and a single gavage administration on Day 15. The drinking water and pre-conditioning gavage target dosages (mg/kg) were 2.88, 28.8, or 57.6 for the male rats, 2.74, 27.4, or 54.9 for the female rats, 8, 80, or 160 for the male mice, and 10, 100, or 200 for the female mice. Plasma samples were analyzed for BCA concentration using a validated capillary GC with electron capture detection method. A one-compartment model was used to describe the plasma concentration time profiles. For the rats, the rate of BCA elimination was decreased and the dose-normalized AUC was increased following the pre-conditioning. For the mice, the elimination rate was not substantially altered whereas the dose-normalized AUC was increased due to the pre-conditioning. The results of this study indicated that the kinetics of BCA was affected by repeated exposure, which led to a decrease in the elimination of BCA, except for the mice, and an increase in systemic exposure to BCA. This study was supported by NIH (N01-ES-5456).

973 HUMAN GLUTATHIONE S-TRANSFERASE OMEGA IS INHIBITED BY TOCOPHEROL ESTERS.

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Human glutathione S-transferase omega 1-1 (hGSTO1-1) is a newly identified member of the glutathioneS-transferase (GST) family of genes, which also contains alpha, mu, pi, sigma, theta, and zeta members. These enzymes contribute to the biotransformation of a wide range of exogenous and endogenous compounds. hGSTO1-1 catalyzes the reduction of arsenate, monomethylarsenate (MMA(V)),and dimethylarsenate (DMA(V)) and exhibits thioltransferase and dehydroascorbate reductase activities.
Recent evidence has show that cytokine release inhibitory drugs, which specifically inhibit interleukin-1b (IL-1b), directly target hGSTO1-1. We found that (+)-α-tocopherol phosphate inhibit hGSTO1-1 in a concentration-dependent manner with IC50 of 2 μM. A Lineweaver-Burk plot monstrated the uncompetitive nature of this inhibition. The molecular mechanism behind the inhibition of hGSTO1-1 by α-tocopherol esters (vitamin E) is important for understanding neurodegenerative diseases, which are also influenced by vitamin E.
A wide range of cell culture, animal model, and human epidemiological studies have suggested a role for vitamin E in brain function and in the prevention of neurodegeneration; however, the underlying molecular mechanisms of this activity remain largely unknown.

974 KINETICS FOR ETHYLENE DICHLORIDE (EDC) AND ITS EFFECTS ON LIVER, KIDNEY AND LUNG GLUTATHIONE (GSH) IN RATS FOLLOWING INHALATION OR ORAL ADMINISTRATION.

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EDC is a widely used industrial solvent. Groups of rats (n=3) were exposed to EDC via inhalation (200 ppm, 6 h/day) or gavaged (150 [corn oil, CO] or 43 [water] mg/kg/day) for 1, 3 or 5 days, euthanized at different times during exposure and post-exposure and blood EDC and liver, lung and kidney GSH determined. Steady-state blood EDC concentrations were reached after ~1 h of inhalation, declining post-exposure to non-quantifiable (NQ) levels within ~2 h; the kinetics of EDC in blood were unchanged after repeated exposures. Orally dosed EDC was rapidly absorbed (blood Cmax at 15–30 min) and concentrations were similar between single and repeated exposures indicating that EDC did not accumulate upon repeated exposure. EDC was cleared from blood at different rates for each vehicle (NQ by 2 h [water] and by 8-16 h [CO]). Liver GSH was decreased during both single and repeated inhalation exposures, but the decreases were more pronounced (58% of control values) on the 1st day of exposure as compared to the subsequent days (82-94% of control values), likely due to a rebound "over-synthesis" of GSH between exposures. Liver GSH after 1 day of exposure rebounded to control levels at 2 h post-exposure and recovered to nearly control levels (~85%) at 2 h post-exposure for animals exposed repeatedly. EDC in CO caused similar decreases in liver GSH trending downward at all times post-dosing, persisting up to 8 h (35-50% of the controls). The decrease in liver GSH produced by EDC in water was 65-77% of controls and returned to control levels by 8 h post-dosing. These differences may be a result of either a dose-dependency in EDC kinetics or vehicle effect. EDC by inhalation or oral dosing had minimal effect on kidney and lung GSH levels (decreases of 1-15%). Regardless of the route of administration, EDC was rapidly eliminated from the body and repeated exposures had no impact on the kinetics of EDC in blood or the depletion of GSH in tissues. Funded by the HAP Task Force, consortium of US EDC producers.

975 *IN VITRO* METABOLISM OF NIMESULIDE: INVESTIGATION OF ITS HEPATOTOXICITY.

F. Li, M. Chordia and T. Macdonald. *Chemistry, University of Virginia, Charlottesville, VA.* Sponsor: C. Holstege.

Nimesulide (Nim), a well-known cyclooxygenase-2 inhibitor, is one of the nonsteroidal anti-inflammatory drugs (NSAIDs) marketed in more than 50 countries except the United States. This drug displayed approximately 100 adverse effects per 100,000 people in comparison to other NSAIDs, which has as few as 1 case per 100,000 people in post marketed studies and thus it has been plagued with severe adverse effect. The drug was withdrawn in Finland and Spain due to its hepatotoxcity. 4-Amino-2-phenoxy-methanesulfonanilide (M1), a reduced metabolite of NIM (in vivo), was reported in several publications. We hypothesized that M1 is a stable metabolite susceptible to facile oxidation by P450 to form very reactive diminoquinone metabolite (M2) which may be responsible for covalent modification of biomolecules. Experiments performed with Human liver microsome incubation reveals a conjugation species from M1 and GSH or NAc, and this conjugation is in a P450-dependent manner. Human serum albumin is an abundant protein in plasma. The alkylation of M1 to HSA is also studied. The conjugation competition of HSA and GSH to M1 may reveal nim hepatotoxicity.

976 EVALUATION AND ACCREDITATION OF INTERNATIONAL ANIMAL CARE AND USE PROGRAMS.

J. Swearengen. *AAALAC International, Frederick, MD.* Sponsor: L. Koller.

The trend of conducting animal studies overseas by pharmaceutical companies and the growth of international collaborative studies involving animal use within academia have led to an increase in international interest in accreditation. Within the United States, misunderstanding still exists regarding the process which AAALAC International uses for accrediting animal care and use programs in other countries. The objectives of this presentation are to clarify the methodology used by AAALAC International to evaluate animal care and use programs outside of the United States. Expectations for both physical plant and programmatic perspectives will be included. This review will include a discussion of the standards and reference resources applied to programs outside of the United States and specific examples will be provided of how the Guide for the Care and Use of Laboratory Animals (Guide)(ILAR, 1996) is applied in context of existing national or local legislation. Understanding the process and standards used to evaluate animal care programs in countries where existing animal welfare standards may be considerably different from those in the United States is important to those involved in establishing collaborations in other countries and to those concerned that an equivalent level of animal care is being provided, for both scientific and welfare purposes.

977 THE USE OF "CLEAN" FUELS TO IMPROVE AIR QUALITY.

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Several air quality districts have promoted "Clean" fuels such as compressed natural gas (CNG) as a means to reduce vehicle emissions and improve air quality. To see if this regulatory approach will produce lower emissions, we compared emissions from vehicles fueled with diesel or CNG giving attention to the impact of currently available engine exhaust after treatment technology on emissions. Emissions data was collected from 25 reports on transit buses, school buses, refuse trucks, and passenger cars. The data was organized by vehicle type and exhaust after treatment and was analyzed using standard analysis of variance statistical procedures. For vehicles without engine exhaust after treatment, CNG vehicles had the highest emissions of carbon monoxide, hydrocarbons, non-methane hydrocarbons, volatile organic compounds (e.g. benzene, butadiene, ethylene, etc.) and carbonyl compounds (e.g. formaldehyde, acetaldehyde, acrolein). Diesel vehicles without exhaust afterment had the highest emissions of particulate matter, and polycyclic aromatic hydrocarbons. For the most part, nitrogen oxides and carbon dioxide emissions were unaffected by exhaust after treatment. The application of exhaust after treatment technology reduced most emissions to similar levels between diesel and CNG fueled vehicles. Since emissions were similar between diesel and CNG fueled vehicles equipped with current exhaust after treatment technology, giving preference to one fuel does not appear to be an effective approach for reducing vehicle emissions and improving air quality.

978 THE APPROPRIATENESS OF THE USE OF THERAPEUTIC DRUGS ON REGULATORY TOXICOLOGY STUDIES AND ETHICAL CONSIDERATIONS.

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Historically there has been reticence to administer therapeutic drugs to animals on toxicology studies for fear of adversely affecting the study outcome. Occasionally, however, drugs to alleviate pain or inflammation or to treat general symptoms of disease should be administered as study animals should be alert, active and healthy. All logistics behind animal health ensure that only animals in good health are allocated to study. It is also clear that during a toxicology study incidents occur, such as damage to extremities, lesions and general ill health, not related to test article toxicity, that require intervention.

Our objective is to explore the issues surrounding the humane treatment of animals to ensure that they are treated with appropriate therapies when the unforeseen occurs. These include the necessity of not administering drugs needlessly, trying the least invasive methods first and appropriately researching the likelihood of drug interactions with the test article.

The Clinical Veterinarian (CV), technical staff, Study Director and Sponsor play pivotal roles in this process. There must be interaction between each of the members of the group to ensure appropriate actions are taken when the need arises. There are also ethical concerns governing what is/is not appropriate when dealing with animals. Euthanasia rather than treatment is sometimes the best option to relieve suffering.

The CV's inherent responsibility includes monitoring and providing recommendations concerning: preoperative procedures, surgical techniques, qualification of institutional staff performing surgery and providing postoperative care. Examples of classical vs. conservative treatments are listed together with the logistics of the process and the information flow between scientific personal and the CV.

The cornerstone of the whole processes is to ensure reliable data from the studies whilst respecting animal welfare.

979 MANGANESE AND THE DEVELOPMENT OF REFERENCE EXPOSURE LEVELS TO PROTECT AGAINST 8-HOUR AND CHRONIC EXPOSURES.

B. Winder[1], D. Morry[2], A. Salmon[2] and M. Marty[2]. [1]*OEHHA, CalEPA, Sacramento, CA and* [2]*OEHHA, Cal/EPA, Oakland, CA.*

Long term manganese inhalation can lead to a characteristic neurotoxicity known as manganism whose manifestations may include motor deficits (dystonia, altered gait, fine tremor, generalized rigidity) and psychiatric disturbances. At low manganese levels, subtle deficits in cognitive and neurobehavioral functions have been reported in both adults and children. Neurodevelopmental deficits have been associated with early life exposure to excessive manganese and include impaired intellectual performance and behavioral disinhibition. To protect susceptible populations, OEHHA has developed reference exposure levels (RELs) for periodic 8-hr, and chronic inhalation exposures to manganese based on neurobehavioral deficits in adults following occupational exposure (Roels et al., 1992). A LOAEL of 150 μg/m3 was reported for impaired visual reaction time, eye-hand coordination, and hand steadiness. Because manganese is a developmental toxicant and may bioaccumulate in infants (who lack Mn homeostasis), this value was adjusted to reflect a continuous exposure to 107 μg/m3 for the 8-hr REL, while a time-adjusted value of 54 μg/m3 was used for the chronic REL. An uncertainty factor (UF) of 6 was used for the absence of a NOAEL, and √10 for the use of a subchronic study. To protect children, a toxicokinetic UF of 10 addresses the greater particle deposition in children's lungs, and a toxicodynamic UF of 10 reflects the neurodevelopmental susceptibility of children. The cumulative UF for both was 2000 giving an 8-hr REL of 0.05 μg/m3, and a chronic REL of 0.03 μg/m3.

980 THE DEVELOPMENT OF REFERENCE EXPOSURE LEVELS TO PROTECT AGAINST ACUTE, 8-HOUR AND CHRONIC EXPOSURES TO ACROLEIN.

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Acrolein is a sensory irritant at low exposure levels. However, asthma exacerbation and, with longer exposure, lesion formation in respiratory epithelia have also been observed. While most acrolein in the ambient air results from atmospheric reactions between butadiene and ozone, acrolein also derives from the combustion of organic matter. To protect susceptible populations, OEHHA has developed reference exposure levels (RELs) based on sensory irritancy in humans, and lesion formation in rat respiratory epithelium. In human studies, LOAELs for ocular irritation of 0.06 ppm (Darley et al., 1960), and 0.07 ppm (Weber-Tschopp et al. 1977) were reported. In both cases an uncertainty factor (UF) of 6 for the use of a LOAEL, and a toxicodynamic UF of 10 to account for potential asthma exacerbation in children, were applied giving acute RELs of 2.3 μg/m3 (1.0 ppb), and 2.7 μg/m3 (1.2 ppb), respectively. These values are supported by a benchmark concentration (BMC05) of 56 μg/m3 for lesions in rat respiratory epithelium (Cassee et al., 1996). Extrapolation to humans used a dosimetric adjustment factor (DAF) based on a computational fluid dynamics model (Kimbell et al., 2001). An interspecies toxicokinetic UF of 2 was used with the DAF, and in the absence of interspecies data, a toxicodynamic UF of √10. A toxicodynamic UF of 10 addressed the for potential asthma exacerbation in children. The resulting REL of 2.1 μg/m3 (0.91 ppb) supports the acute REL of 2.3 μg/m3 based on human studies. The 8-hr and chronic RELs are based on the occurrence of lesions in the respiratory epithelia of acrolein-exposed rats (Kutzman et al., 1985). Extrapolation from 6-hr to 8-hr exposures was achieved with a modification of Haber's Law. Extrapolation to humans used the DAF and an interspecies toxicodynamic UF of 2. As above, the cumulative UF values include a 10-fold toxicodynamic uncertainty factor to address the greater impact of acrolein exposure on children and asthmatics. This resulted in an 8-hr REL of 1.6 μg/m3 (0.68 ppm), and a chronic REL of 0.1 μg/m3 (0.05 ppm).

981 IMPLEMENTING THE CALIFORNIA SAFE COSMETICS ACT OF 2005.

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Consumers, workers, and public health advocates seeking more information about ingredients in cosmetics products supported the passage of the "California Safe Cosmetics Act of 2005." Cosmetics contain many chemicals, often as mixtures in complex formulations. Some of these chemicals are known or suspected to be carcinogens, teratogens, or developmental toxicants. However, the risk that cosmetics may pose to users is generally unknown because of limited information on exposure or lack of scientific studies on potential health effects. Hair stylists, nail salon workers, and others who work in cosmetics, skin and body care may be at increased risk for adverse health effects from using these products because they handle greater quantities of them, and with greater frequency. For all cosmetic products sold in California, the Act requires the manufacturer, packer, and/or distributor named on the product label to provide to the California Department of Public Health (CDPH), on a schedule determined by CDPH, a list of all cosmetic products that contain any ingredients known or suspected to cause cancer, birth defects, or other reproductive harm. This requirement does not apply to any company with annual aggregate sales of cosmetic products, both within and outside of California, of less than $1 million. Although companies are not exempt from reporting trade secret information, the law does not allow this information to be disseminated to the public. The purpose of the California Safe Cosmetics Program (CSCP), which was created in CDPH to implement the provisions of the Act, is to collect chemical data and other information on the ingredients in cosmetic products, make it available to the public, and conduct health investigations as resources permit. CSCP has compiled a list of chemical agents for disclosure according to the legislative specifications, and is currently developing a reporting system and a database of product ingredient information submitted by companies. A summary of the regulatory and scientific bases as well as the challenges and opportunities for implementing the Act will be presented.

982 CHEMICALS RISK ASSESSMENT UNDER THE NEW EUROPEAN REACH REGULATION.

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REACH (Registration, Evaluation, and Authorization of Chemicals) is the European Union's new Regulation for the management of risk to human health and the environment posed by chemical substances. At its core, REACH will require producers and users of chemical substances to register these uses in a volume-triggered system. It demands the submission of chemical assessment reports (CSR) containing information on the hazards, exposures and risks associated with the uses of chemical substances for review by the competent authorities and government-appointed expert committees. Chemicals deemed to be of very high concern, e.g., carcinogens, mutagens or substances toxic to reproduction, will trigger a complex authorization procedure in which the ultimate political goal is to substitute those chemicals with safer alternatives. After more than six years of scientific and political debate it came into force on June 2007. The European Chemicals Agency (ECHA) which is tasked to manage the REACH process will become operational in June 2008 at which time the pre-registration process will begin. The first deadline for registration of high volume chemicals and those considered to be of very high concern has been set for end 2010. This paper will characterize and discuss the REACH risk assessment process as specified in the REACH guidance documents. It will give specific focus on data requirements and the iterative development of the

chemical safety assessment (taking into account existing risk management measures) using the IUCLID5 tool. This includes the development of exposure scenarios and the derivation appropriate no effect level or minimal effect levels depending on exposure scenario (e.g., acute versus chronic) and hazard profile (e.g., threshold versus non-threshold) as well as the risk characterization process. The paper further discusses the possibilities for data waiving and the use of alternative methodologies such as in vitro testing, (Q)SARs (structural activity relationships) or read-across approaches.

983 INTEGRATION OF LIFE-STAGE AND EXPOSURE DURATION ASSESSMENTS INTO DERIVATION OF STANDARDS.

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The Minnesota Department of Health (MDH) establishes human health protection values called "Health Risk Limits" (HRLs). HRLs are expressed as micrograms of contaminant per liter of groundwater (ug/L) and represent a concentration that is without appreciable risk to human health whether ingested only once or daily for a lifetime. Historically, HRLs were thought of as protecting against adverse health effects from chronic exposure and combined an adult intake rate (2 L/day and 70 kilogram body weight or approximately 29 mL/kg-day) with a chronic reference dose. As part of the current rule revision MDH incorporated a variety of risk characterization approaches as recommended by the EPA Review of the Reference Dose/Reference Concentration Processes report. The evaluation for deriving non-cancer HRLs included: 1) evidence of life stage sensitivity; 2) assessing the relationship between the effects observed and duration of exposure; 3) selecting endpoints based on characterization of the entire database rather than the "critical" study; 4) incorporation of water intake rates based on age and duration considerations; and 5) evaluating the range of potential HRL values for different duration reference values and exposure durations before selecting a final value. The evaluations demonstrated that the historic reliance on chronic assessments may not be protective of less-than-chronic exposures. The result was a range of HRL values that ensure protection of sensitive life-stages and periods of high exposure. Evaluation of shorter durations lead to the identification of precursor events for chronic effects as well as endpoints attributed to shorter exposure periods. The major challenge to employing these methods was insufficient data for many of the chemicals of concern. Current testing protocols are typically not designed with a focus on assessing different life stages (timing of exposure) or different durations of exposure, therefore, these factors are often not well characterized. Examples of evaluations for specific PFCs, VOCs and pesticides will be presented.

984 TOXICOLOGY CONSIDERATIONS FOR PRECLINICAL DEVELOPMENT OF ANTICANCER DRUGS.

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There is no universally accepted approach in the development of anticancer drugs. This is the consequence of the lack of a specific guideline as only regional ones or recommendations are followed in different geographical areas. If on the one hand, this may suggest some freedom to operate, on the other this lack of harmonization creates difficulties in having programmes accepted worldwide without potentially unnecessary use of resources and laboratory animals.
Additionally, the approach to the pre-clinical development of anticancer drugs is also depending on the class of drugs under investigation e.g. small molecules versus biological drugs. Furthermore, the approach within a given class of compounds is dependent from their respective mechanism of action. As an example, the pre-clinical development of a small molecule having cytotoxic properties requires different strategies with respect to a non-cytotoxic compound (e.g. anti-angiogenic compounds, hormonal drugs). Considering the high unmet medical need in the field of oncology drugs, it becomes more and more important to ensure a harmonized approach in order to make available new therapies to patients with shorter development programs, well-defined study typologies allowing minimization of animal use. With this aim, the International Conference of Harmonization (ICH) has initiated the process of preparing a guideline (ICHS9) that aims to address all the main points. This guideline should become effective from 2010.
Working for years in the pre-clinical development of anticancer drugs, Accelera has acquired a wide expertise in this field. Specific approaches used for the pre-clinical development of cytotoxic and non-cytotoxic compounds are exemplified on the basis of general toxicology, safety pharmacology and other in vitro/in vivo tests.
New perspectives and scenarios based on current trends and on the forthcoming ICH guideline are also mentioned.
Reference:
- "S9 Preclinical Guideline on Oncology Therapeutic Development" Final Concept Paper; April 30, 2007

985 PROGRESSING FROM THE MOUSE BIOASSAY FOR SHELLFISH TOXIN TESTING IN CANADA.

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Sponsor: A. Goldberg.

Bivalve mollusks are filter-feeders that accumulate marine biotoxins which can severely affect the health of those who ingest them. To ensure the safety of its shellfish, the Canadian Food Inspection Agency manages a marine biotoxin monitoring program that relies on a mouse bioassay. While Canadians expect the government to protect public health, they rely on the Canadian Council on Animal Care, Canada's oversight body for the care and use of animals in science, to ensure that the use of animals in testing employs optimal physical and psychological care without compromising scientific integrity. The continued use of the mouse bioassay is ethically challenging because of the potential for extreme pain and distress. Canadian research has led to the development of non-animal test methods that are more sensitive and more reliable than the mouse bioassay, but these have not yet been adopted by Canadian regulators despite international validation studies. This research study used a policy stream model to characterize the problems and the solutions involved in the adoption of alternative methods for shellfish toxin testing. An ethnography study of stakeholders in shellfish toxin testing revealed that Canadian regulators still use the mouse bioassay because none of the alternative methods have been accepted as standards for international trade. Problems identified include: the incomplete set of certified reference standards; the insufficient throughput of liquid chromatography-based methods; and the high cost of the equipment for developing countries. Solutions identified include: research to develop more reference standards; improvement in the throughput of alternative methods; and the adoption of a two-tiered testing system for First World and Third World countries. Looking for opportunities to act where the problem stream and the solution stream converge, this study points to a need for more focus to be placed on validation of existing methods, rather than on further research on the development of new instrument-based methods, in order to balance protection of public health and ethical animal use.

986 EXTRAPOLATING HUMAN EFFECT THRESHOLDS FROM ANIMAL TOXICITY DATA FOR THE DERIVATION OF IMMEDIATELY DANGEROUS TO LIFE AND HEALTH VALUES.

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Immediately Dangerous to Life or Health (IDLH) values have a long history of use in industrial settings in defining work practice and respiratory protection requirements for entry into potential high exposure environments. The National Institute for Occupational Safety and Health (NIOSH) defines the IDLH values as "airborne concentrations of a substance that may, in an occupational inhalation exposure of 30 minutes or less, pose an immediate threat to life, or cause irreversible adverse health effects, or interfere with the capability of a worker to escape should respiratory protection fail." This study was conducted to 1) critically assess the current strategy for the derivation of new IDLH values, 2) compare IDLH values derived from non-lethal toxicity data versus those derived from acute lethality data, and 3) examine the differences in the ratios of animal-to-human effect levels for various modes of action. As an update to a prior analysis (Weinrich et al., 2005), the acute toxicity data for 20 new high priority compounds (i.e., chemical terrorism agents, industrial or agriculture chemicals agents, or other agents that cause serious health effects from acute exposures) was assembled and provisional IDLH values were derived using alternative approaches. Alternative methods for extrapolating from acute lethality data in animal studies (LC50, LC10, and lowest lethal concentrations) to human exposure thresholds were compared. The findings indicate mixed results with a significant mode of action effect observed for some of the 20 chemicals. Overall, this work further enhances the transparency of the underlying rationale for the methods used to derive IDLH concentrations and provides a basis for updated safety factor selection. Lessons learned from this research were used in the derivation of new IDLH values for chemicals of interest to homeland security applications.

987 DEVELOPMENT OF AN IMPROVED STRATEGY FOR THE DERIVATION OF SKIN NOTATIONS.

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Contact of the skin with chemical substances represents a significant route of exposure within workplace settings. The occupational safety and health community has relied on skin notations to identify and communicate the health hazards associated with dermal chemical exposures since the 1950s. These skin hazard designations have traditionally been used to indicate that a chemical has the potential to be ab-

sorbed through the skin and contribute substantially to systemic toxicity. Despite their importance as a risk management tool, several shortcomings have been identified with their continued use in their current form. The primary limitations are 1) the failure of the warnings to be assigned based on a standardized methodology, and 2) their inability to provide a warning for localized and sensitizing effects of chemical exposures. The National Institute for Occupational Safety Health (NIOSH) has developed a new strategy for the assignment of improved skin notations designed to address these issues. This process is a form of health hazard identification that provides the scientific rationale and framework for the derivation of multiple skin notations that clearly distinguish systemic, direct, and sensitizing effects caused by dermal exposures to chemical substances. This method relies on a critical evaluation of reports of human exposure and health effects, empirical data from animal toxicity studies, considerations based on mathematical models, and the application of a weight-of-evidence approach to assess the health risks associated with chemical contact with the skin. Issues encountered during the derivation of skin notations for 48 chemicals are presented. Special emphasis is placed on the application of the scientific rationale, the use of toxicological cut off values to determine systemic toxicity, and data quality.

988 THE NICEATM-ICCVAM FIVE YEAR PLAN: CREATING A PATH FORWARD TO REDUCE, REFINE AND REPLACE ANIMAL TESTING.

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NICEATM and ICCVAM have developed a five-year plan that builds on the mission, vision, and strategic priorities in the 2004 ICCVAM Strategic Plan. Implementing the five-year plan involves four key challenges. The first is to identify priority toxicity testing areas for the next five years, and to conduct and facilitate activities in those areas. Currently, the four highest-priority areas are ocular, dermal, acute, and biologics testing. Other priority areas include immunotoxicity, endocrine disruptors, pyrogenicity, reproductive and developmental, and chronic toxicity/carcinogenicity testing. The second challenge is to identify and promote research initiatives that are expected to support future development of innovative alternative test methods. These new methods might incorporate techniques such as high throughput screening, computer modeling, informatics, and biomarkers. The third challenge is for NICEATM and ICCVAM to foster the acceptance and appropriate use of alternative test methods through outreach and communication. This will be accomplished by sponsoring and participating in workshops, NICEATM-ICCVAM website communications, and the development and publication of standardized test method protocols. Finally, ICCVAM and NICEATM will develop partnerships and strengthen interactions with stakeholders to facilitate meaningful progress. These efforts are expected to facilitate research, development, translation, validation and regulatory acceptance of alternative methods that will reduce, refine, and replace animal use while maintaining scientific quality and the protection of human health, animal health, and the environment. Contract support provided by NIEHS contract N01-ES-35504.

989 CREATION AND IMPLEMENTATION OF DATA RESOURCES SUPPORTING INTEGRATED SCIENCE ASSESSMENTS OF CRITERIA AIR POLLUTANTS.

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Under the Clean Air Act (CAA) as amended in 1990, the US Environmental Protection Agency (EPA) is charged with establishing and periodically reviewing National Ambient Air Quality Standards (NAAQS) for six criteria pollutants: carbon monoxide, ozone, nitrogen dioxide, particulate matter, sulfur dioxide, and lead. The CAA requires EPA to review and, if appropriate, revise the NAAQS for each pollutant at five year intervals. A high level of effort is needed to adequately review and integrate the science (e.g., air quality analyses, quantitative risk/exposure assessments, risk characterization) that informs the Agency decision-making process. EPA recently decided to change the process of revising the NAAQS to help meet the mandated 5-year schedule. Included in this set of revisions is development of integrated science assessment (ISA) documents to address the most policy-relevant science. Timely development of the ISA will be facilitated by the use of a continuous process for identifying, compiling, characterizing, and prioritizing relevant new scientific studies into a database to store, organize and allow analysis of the scientific basis for each NAAQS. This "evergreen" database would encompass the entire breadth of information used in assessing the state of the science on exposure, ecological effects and health effects (epidemiologic, clinical, and toxicologic). This effort is also anticipated to enhance other data sharing activities within the EPA and in collaborations with other agencies and organizations. Examples of analyses, reports, queries and graphics created using the database thus far developed, and their relationship to the ISA documents will also be presented.
[The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.]

990 THE USE OF HEALTH-BASED OCCUPATIONAL EXPOSURE LIMITS AS REACH DERIVED NO EFFECT LEVELS.

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Under the European Union's new Registration, Evaluation, and Authorization of Chemicals (REACH) regulation, Derived No Effect Levels (DNELs) represent a level of exposure above which humans (e.g. consumers, workers, etc.) should not be exposed. In risk characterization of REACH-regulated chemicals, known and/or potential exposures are compared to the appropriately derived DNEL. The health risk is considered adequately controlled if the exposure does not exceed the DNEL. Compliance with traditional health-based occupational exposure limits (OELs) has been one of the most significant and useful methods for ensuring worker health and safety. As REACH stands right now, even though there may be an authoritative health-based OEL(s) (e.g. ACGIH TLVs, AIHA WEELs, German MAK, SCOEL, etc.) for a particular REACH chemical, a DNEL still needs to be derived (i.e. for long-term occupational exposure via inhalation) as the "benchmark" for exposure comparison. Because of current differences in the methodologies for deriving DNELs and OELs (e.g. in the use of professional judgment and experience, assessment, uncertainty/safety factors, etc.) it is generally anticipated that for any particular chemical, the health-based OEL will differ from that of the REACH-derived DNEL. Moreover, some experts believe that the workplace inhalation DNEL will be lower than its corresponding health-based OEL. Therefore, it is possible that workers, health and safety personnel (e.g. toxicologists, industrial hygienists, etc.), and managers will be confronted with both a DNEL and an OEL for the same chemical on which they need to make risk management (e.g. exposure reduction) decision. This poster will present background on the development of both DNELs and health-based OELs, discuss some of the potential regulatory and health & safety issues/consequences regarding the concurrent development and use of a DNEL and OEL for the same chemical in the occupational setting, and provide an overview of any updates regarding the REACH regulation (e.g. to the RIP 3.2-2 TGD) as it applies to the use of OELs as DNELs for workplace exposures.

991 SYSTEMIC TESTING BY THE DERMAL ROUTE CAN BE PRECLUDED BY *IN VITRO* OR *IN SILICO* PERCUTANEOUS ABSORPTION STRATEGIES.

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Recent developments in percutaneous absorption testing now make it possible to determine, using either in silico or in vitro prediction methods, to what degree a specific chemical will be absorbed through the skin. If there is concern for exposure by the dermal route but chemicals have little or no dermal penetration potential, systemic toxicity tests can be avoided. Within programs such as the US EPA's High Production Volume chemical challenge, this approach can be particularly rewarding. Here we present data generated using the QSAR model EPA DERMWIN for the mixture Commercial Hydroxyethyl Piperazine (CHEP), sponsored in the HPV program by The Dow Chemical Company. To check the applicability of the model experimental and modeled absorption data for ten analogous amines were compared; the ratio of experimental to modeled data was of acceptable concordance (values ranged from 0.1 to 10.5) and the absorption of CHEP was modeled. The components were predicted to minimally absorb through the skin, with estimated average total absorption at 1.99 mg/kg/day. When considered together with other factors such as production and use, protective equipment, and available data on piperazine (the component predicted to penetrate in the largest amount), this estimation supported the conclusion not to conduct a stand-alone dermal reproductive and developmental toxicity study. This approach protects public and worker health while avoiding resource- and animal-intensive tests such as dermal developmental or reproductive toxicity.

992 AUTOMATED SELF-SIMILARITY ANALYSIS OF AIRWAYS IN THE SPRAGUE DAWLEY RAT.

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The rat is extensively used as an animal model for respiratory disease and inhalation toxicity. A quantitative description of the rat lung morphometry is therefore foundational to mathematical models of gas-exchange and particle deposition in the rat, and forms the basis for realistic boundary conditions for multiscale models of rat lung mechanics. A morphometric analysis across animals can also define a distribution of important morphometric parameters and thus help to define a 'normative' animal upon which to base generalizations of experimental and computational findings. We developed an automated method for tabulating morphometric data from magnetic resonance images of the rat bronchial tree, and a succinct morphometric framework for comparisons among animals. This method was validated against hand measurements of lung casts. Once airway geometries were segmented from MRI data, morphometric and statistical analysis were performed in under five minutes per lung. Computer-generated measurements of airway diameters averaged 0.13 mm (95% C.I. -0.16 mm to -0.09 mm) smaller than the corresponding measurements obtained manually, while airway lengths averaged 0.13 mm longer, but the difference was not statistically significant. Fits to the six variable parameter vector for 28 'branches' per lung were excellent and in agreement with the parameterization of the manual measurements. Parameterization of the left and right principle paths down to the terminal bronchioles, was predictive of the lung as a whole. Comparisons among a growing library of rat lung geometries have established a normative geometry of the Sprague Dawley bronchial tree. This method is also useful for in vivo hyperpolarized 3He gas imaging studies to characterize the compliance of the entire tree from the few airways resolvable with this modality to improve 3D model development under toxicant-induced disease conditions. Funded by NHLBI RO1 HL073598-01

993 SIMULATION OF INSPIRATORY AIRFLOW IN THE B6C3F1 MOUSE NASAL PASSAGES.

J. S. Kimbell, E. A. Gross and J. D. Schroeter. *The Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

Studies of nasal lesions induced by inhaled gases such as chlorine, formaldehyde and hydrogen sulfide routinely include data from both rats and mice. Anatomically accurate computational fluid dynamics (CFD) models of the F344 rat nose have been previously made to correlate lesion incidence and localized tissue dose. These correlations and human CFD models have enabled risk estimation in humans. Confidence in these methods will be increased if the CFD modeling approach predicts similar correlations in the mouse. A three-dimensional reconstruction (3D-R) of both sides of the nasal passages of an adult, male B6C3F1 mouse (approx. 30 g) was constructed from digital photographs of 50-µm serial-step sections through a minimally-fixed, frozen tissue specimen. The locations of squamous, transitional, respiratory, and olfactory epithelium were mapped into the 3D-R using light microscopy and Mimics (Materialise, Ann Arbor, MI). A tetrahedral mesh with 2.5 million cells was generated using ICEM-CFD (ANSYS, Canonsburg, PA). Steady-state inspiratory airflow was simulated using Fluent (Fluent, Inc., Lebanon, NH) at an estimated minute volume of 24.5 ml/min by imposing a pressure drop between the nostrils and nasopharynx of 65 Pa. Major routes of predicted airflow were similar to those in the rat and were located anteriorly in dorsal, middle lateral and middle meati with lesser streams in dorsal medial and ventral medial meati. Posteriorly, major airflow streams were located in the ventral medial meatus with lesser streams forming Z-shaped patterns adjacent to the dorsal septum and medial aspects of the first and third ethmoturbinates. Countercurrent backflow was observed adjacent to the fifth and sixth ethmoturbinates (terminology from Mery et al., Toxicol. Pathol. 22:353-372, 1994). As in the rat, inspiratory airflow passing olfactory-lined areas originated from the dorsal medial meatus anteriorly. These results will provide a means for interspecies comparison of predicted correlations of tissue dose and response and thereby increase confidence in their use for estimating risks to human health.

994 DOSIMETRY PREDICTIONS OF INHALED HEXAMETHYLENE DIISOCYANATE IN THE HUMAN RESPIRATORY TRACT.

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Hexamethylene diisocyanate (HDI) is a reactive chemical used in the commercial production of polyurethane products. Toxic effects in animals exposed to HDI vapor include respiratory tract irritation, degeneration of olfactory epithelium, and pathological changes in the alveolar airways. Concerns for human exposure to HDI are focused on potential responses of the tracheobronchial or pulmonary regions of the lung, including airway hypersensitivity and reversible reductions in lung function, with the main health concern being the potential of the isocyanate to initiate occupational asthma. In this study, a dosimetry model was developed to predict localized tissue dose of inhaled HDI vapor in the human respiratory tract for nasal or oronasal breathing. The model consisted of an anatomically accurate computational fluid dynamics model of the human nasal passages that was linked to a lung dosimetry model. Airflow and HDI transport in the nasal passages were simulated using the finite element software FIDAP (Fluent, Inc.). Transport of HDI through lung and oral airways was simulated by convection and axial dispersion and was implemented in MATLAB (Mathworks, Inc.). The nasal and lung components of the dosimetry model included tissue phases where absorption of HDI was governed by diffusion and first-order clearance. Nasal or oronasal breathing was used depending on the inspiratory airflow rate for different activity states. Tissue dose was predicted in the nasal and oral passages and in all generations of the lung. The simulations predicted that the upper respiratory tract and upper bronchial airways received the highest tissue dose, yet there was still penetration of HDI vapor to the pulmonary airways. These tissue dose estimates in human tracheobronchial and alveolar regions can be correlated with corresponding dosimetry predictions in rats that are known to cause adverse responses.

995 NANOPARTICLE DEPOSITION IN THE RAT NASAL CAVITY: PREDICTION OF DOSE TO THE OLFACTORY EPITHELIUM.

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Potential health risks associated with inhalation of nanoparticles are mostly unknown. A recent study in rats showed that nanoparticles depositing in the olfactory region can translocate along nerve axons to the olfactory bulb in the brain (Oberdoerster et al., Inhal. Toxicol. 16:437-445, 2004). An accurate estimate of the dose of inhaled nanoparticles to the rat olfactory epithelium will assist in understanding potential toxic effects and extrapolating such findings to humans. The goal of this study was to quantify nanoparticle deposition in the rat nasal cavity and, more specifically, the olfactory region. A three-dimensional, anatomically-accurate computational fluid dynamics model of the rat nasal airways was developed. A tetrahedral mesh was created in ICEM-CFD (Ansys, Inc.) with major epithelial types mapped onto its surface. Airflow and nanoparticle transport were simulated using Fluent software (Fluent, Inc.) for airflow rates corresponding to 1, 1.5 and 2 times the resting minute volume of 288 ml/min and particle sizes from 1 to 100 nm. For these particle sizes, particle deposition is dominated by diffusion, thus an Eulerian approach was used in the computations. Simulations predicted that nasal deposition decreased with increasing particle size: more than 90% of 1-nm particles and less than 1% of 100-nm particles were filtered by the nose. Olfactory deposition was maximal for particles with diameters of 2-6 nm, with 5 to 9 % of the inhaled particles deposited in the olfactory region. Deposition fractions were somewhat influenced by airflow rate, with higher airflow leading to (1) fewer particles being extracted by the nose and (2) a shift in the spatial distribution of deposited particles, with more deposition in the olfactory region. These results will help decrease uncertainty associated with estimates of potential health risks from inhaled nanoparticles. (Supported by CDC/NIOSH #000HCCEE-2006-36673.)

996 A MODEL OF NANOPARTICLE TRANSPORT AND DEPOSITION IN THE NASAL AND LUNG AIRWAYS OF HUMANS AND RATS.

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Recent studies have suggested that airborne nanoparticles may pose a greater threat of toxicity merely due to their small sizes. With increasing production, there is a concern over the health effects of inhaled engineered nanoparticles. Functional relationships of nasal deposition fraction of nanoparticles as a function of physical and physiological parameters were developed and fitted to deposition measurements in nasal airway casts of humans and rats. The resulting semi-empirical models were incorporated in a mathematical model of nanoparticle deposition in the lung that accounted for axial diffusion and dispersion. Model predictions in human and rat lungs were confirmed by comparing with available lung deposition measurements in the literature. In both species, nanoparticles were found to deposit primarily in nasal passages with a small amount entering lung airways. Nearly all particles below 5 nm were deposited in nasal airways. For particles below 10 nm in diameter, lung deposition was confined to upper airways of the tracheobronchial region. Transport and deposition of nanoparticles in the alveolar region occurred

only when particle size exceeded 10 nm. This work was funded by US EPA STAR Grant #RD832531 and the Long Range Research Initiative of the American Chemistry Council.

997 EVALUATION OF PHYSIOLOGICAL PARAMETERS IN ADULT RATS AND MICE FOR POPULATING AN ACCESS DATABASE.

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Accurate physiological parameter values are a critical component of PBPK models, and as such, it is anticipated that a publicly available repository of physiological data would be a valuable tool for characterizing uncertainties within and between models. The objectives of this study were to collect and evaluate physiological data in adult mice and rats, and incorporate this data into a previously developed Microsoft ACCESS database containing physiological parameters for developing animals. Following an extensive and systematic literature search using PUBMED, 369 peer-reviewed publications were retained for evaluation. Each publication was read to identify the specific parameter of relevance to the database (volumes, flows, composition and metabolism) as well as the experimental design/protocol, sampling method, number of samples, statistical analysis, precision/variability, and bias. Quality-verified data records (rats: 2230, mice: 334) on tissue volumes, blood flow rates, tissue composition and clearance (hepatic, renal, pulmonary) in animals representing a range of body weights (rats: 114 – 864 g, mice: 15-48 g) and strains (rats: 11, mice: 15) were retrieved from 124 peer-reviewed publications and entered within the database. In both species, the availability of volume and flow data was greater for kidney, brain and liver than for other tissues. For illustrative purposes, N-weighted means, combined standard deviations, and combined standard errors of various parameters were calculated using values retrieved from the database. The present study has assembled available data on physiological parameters in adult rats and mice to facilitate better definition of uncertainty and variability associated with these parameters for use in PBPK modeling. Disclaimer: The views expressed are those of the authors, and not the U.S. EPA.

998 A HIERARCHIAL ALGORITHM FOR COMPUTING DISTRIBUTIONS OF HUMAN BLOOD: AIR PARTITION COEFFICIENTS (PB) OF TOLUENE.

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Existing mechanistic algorithms use point estimates for blood composition to predict PB required for PBPK modeling. Analysis of the distributions of variability of blood composition data in humans or characterisation of uncertainty associated with them has not yet been undertaken. In this study, we used previously published human data on toluene PB (Pierce et al., 1996; Toxicol Appl Pharmacol. 139: 49-61) in a Markov chain Monte Carlo (MCMC) approach to update blood composition parameters of the mechanistic algorithm for PB (i.e., neutral lipid equivalent, water and protein content of blood). A hierarchical modeling approach was used to describe the variability and uncertainty associated with blood composition parameters. The information on oil: air (Koa; 1.75 ± 0.23, normal) and water: air (Kwa; 1056 ± 66, normal) partition coefficients was used along with prior information on blood composition (normal; mean values obtained from the literature; 10% interindividual variability and uncertainty) and protein: air (Kpa; normal with mean 50 and 200% uncertainty). MCMC simulations were conducted using WinBUGS 1.4 (MRC and Imperial College of Science, Technology and Medicine, Cambridge, U.K.). Blood composition updates were physiologically consistent, with the mean values being similar to priors but with lower variability (0.8227 ± 0.0502 for water and 0.1762 ± 0.0105 for protein fractions), except for the neutral lipid fraction (0.0043 ± 0.0086). Posterior uncertainty on these parameters decreased drastically (0.2% for lipid; 2.9% for water; 0.6% for protein). Posterior estimates of Kpa (160.2 ± 16.5) were consistent with the literature. The results revealed that experimental data contained enough information to facilitate the characterisation of the variability and uncertainty associated with blood composition. The posteriors resulting from the above MCMC analysis may be used along with the physicochemical characteristics of other VOCs to compute the distributions of their PB for developing probabilistic human PBPK models. (Supported by NSERC, Canada).

999 PREDICTING NERVE AGENT TISSUE:BLOOD PARTITION COEFFICIENTS USING ALGORITHMS ACCOUNTING FOR ACCESSIBLE TISSUE FRACTION.

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Pharmacokinetic modeling of neurological agents (NAs) requires partition coefficients (PCs) to describe the transfer of agents from blood to tissue and vice versa. PCs for many NAs have not been determined experimentally due to their hazardous nature. Using the few experimental PCs available for NAs, algorithms to predict PCs were evaluated. Poulin and Krishnan (1995) developed an algorithm for predicting tissue:blood PCs from n-octanol-water partition coefficients (K_{ow}), a parameter readily predicted for NAs based on chemical structure. These algorithms quite poorly described PCs for the more lipophilic agents, indicating the need to optimize predictors for these kinds of compounds. OPs have both lipophilic and hydrophilic ends (amphiphilic), and may not be able to fully penetrate into fat (their "tails" stick out). The log K_{ow} for these kinds of compounds would therefore overpredict their partitioning into tissue lipids, since they would only penetrate the surface layer rather than the whole fat volume. This poster explores the incorporation of accessible tissue fraction into the published algorithm. The accessible fraction, α, was utilized to predict fat:blood, liver:blood and muscle:blood PCs for a preliminary database of 7-12 NAs and organophosphate (OP) pesticides, structurally similar cholinesterase inhibiting compounds. Preliminary results indicate that the accessible volume (α) for fat, liver and muscle tissues is 0.25, 0.8 and 1, respectively.

1000 EVALUATION OF POTENTIAL BIOACCUMULATION OF UNMETABOLIZED COMPOUNDS BY DE NOVO ESTIMATION OF MAMMALIAN PHYSIOLOGICALLY-BASED PHARMACOKINETICS.

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Previous exposure estimate studies have shown that highly lipophilic compounds (log K_{ow} > 5) may accumulate across the food chain. Authors of recent food-web studies have suggested that moderately lipophilic chemicals (log K_{ow} 2-5) of low volatility (log K_{oa} 6-12) also have the potential to biomagnify (increase in chemical conc. across organisms) up to ~100-fold in mammals, assuming no metabolism in the target organism. The hypothesis that metabolism substantially reduces bioaccumulation potential of this class of compound was evaluated by comparison of empirical pharmacokinetics (PK) from laboratory studies vs. predicted dosimetry with a generic PBPK model that excludes metabolism. Empirical bioaccumulation factors (steady-state vs. single-dose) were calculated from published rodent PK studies. Predicted bioaccumulation values were calculated with a generic (PBPK) model, utilizing QSAR-based equations for blood:tissue partitioning, blood:air partitioning and renal elimination. General model structure was validated with several published datasets. Partition coefficient QSARs were validated with empirical data. Reference physiological values for five mammalian species were also included, to allow for rapid model output comparisons between species. Evaluation of model output shows that unmetabolized compounds with log $K_{ow} \leq 5$ and log K_{oa} values ≤ 12 would have a bioaccumulation potentials of < 16 in the rat. Evaluation of published ADME results for numerous compounds shows that metabolism can reduce this bioaccumulation potential by up to 13-fold from that predicted by the PBPK model. While modelling biomagnification across species or bioaccumulation in one species, without metabolic clearance included, affords a worst-case estimate for risk assessment purposes, metabolism needs to be considered for accurate estimation of accumulation potential.

1001 SCANNING TUNNELING MICROSCOPY OF INDOLO[2, 1-B]QUINAZOLIN-6, 12-DIONE (TRYPTANTHRIN) ANALOGUES: EVIDENCE OF ADSORPTION-INDUCED STEREOISOMERIZATION.

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Scanning tunneling microscopy (STM) has the potential to become a powerful tool in chemical toxicology and pharmacology, especially in relation to refinement of Quantitative Structure Activity Relationship (QSAR) models, metabolism studies, and gaining mechanistic insight into the actions of xenobiotics at the molecular level.

This initial work utilized STM to observe monolayers of indolo[2,1-b]quinazolin-6-12-dione (tryptantrin)and several of its analogues at the solution-graphite interface. Formed by physisorption to highly oriented pyrolytic graphite, sub-molecular resolution of the monolayers was achieved allowing elucidation of the primary electronic features of the individual molecules. The adsorbed molecules form rows that are differentiable via the apparent heights of portions of each molecule, from row to row. This variation is attributed to adsorption-induced stereoisomerization of individual molecules.

Our results suggest that previous theoretical modeling of these broad spectrum antiparasitic tryptantrin compounds, based on a planar conformation from bulk solid crystal structures, may be overly simplistic due to the observed stereoisomerization. This work also allows a more accurate look at the electronic characteristics of tryptanthrin analogues considering that the observed data was not generated using a theoretical model.

1002 PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELING OF BROMOCHLOROACETIC ACID.

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Dihaloacetates (DHAs) occur as water disinfection byproducts commonly present in public water supplies leading to widespread exposure. Dichloroacetic acid (DCA), dibromoacetic acid (DBA), and bromochloroacetic acid (BCA) are all metabolized mainly by glutathione transferase zeta (GSTzeta). A single physiologically-based pharmacokinetic (PBPK) model was developed to enhance the understanding of the kinetics of these three structurally similar dihaloacetates. This current effort focuses on bromochloroacetic acid, which is the only one of these three compounds not presently under EPA regulation. The PBPK model has flow-limited compartments representing kidney, liver, stomach, and other aggregated tissues. The model allows for stereospecific treatment of the BCA(+) and BCA(-) isomers due to published evidence of toxicokinetic behavioral differences between them. Elimination of parent and metabolite compounds occurs through urine or biotransformation. The biotransformation of BCA to the metabolites glyoxalate (GXA) and oxalate (OXA) via GSTzeta-mediated metabolism is described in detail, while a secondary metabolic pathway was also investigated. GSTzeta synthesis and degradation as well as endogenous production of GXA and OXA are included in the model. Molecular interactions of GSTzeta with BCA and BCA-derived compounds are modeled including the covalent binding of an intermediary metabolite with GSTzeta which effectively inactivates the enzyme. Suicide inhibition is characteristic of the dihaloacetates. Model parameters not available from the literature were fit to the BCA, GXA, and OXA time-course data available from IV, gavage, drinking water, and in vitro studies. The model-predicted values for AUC of BCA, GXA, and GSTzeta in blood and liver tissues will be examined as dosimetrics that might account for the carcinogenic activity of this compound.

1003 DEVELOPMENT OF A PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODEL FOR THE INHALATION OF 2, 2, 4-TRIMETHYLPENTANE (TMP) IN LONG EVANS RATS.

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TMP (2,2,4-trimethylpentane,"isooctane") is a colorless liquid used primarily in the alkylation of isobutene and butylene reactions to derive high-octane fuels. TMP is released in the environment through the manufacture, use, and disposal of products associated with the gasoline and petroleum industry. TMP leads to tumors in male rat kidneys possibly due to increased nephropathy associated with α2u-globulin hyaline droplet accumulation. Several short-term studies (less than 4 hours) have shown TMP to cause sensory and motor irritations in rats and mice at inhalation exposures greater than 1000 ppm. In order to relate target tissue dose to inhalation exposure levels, we developed a physiologically-based pharmacokinetic (PBPK) model for TMP in rats using in vivo gas uptake experiments. Gas uptake experiments were conducted by exposing male Long Evans rats (80-90 days old) to initial TMP concentrations of 50, 100, 500, and 1000 ppm. The model consisted of compartments for the gas uptake chamber, lung, fat, kidney, liver, muscle, and brain. Physiological parameters were fixed from literature. Partition coefficients for the model were experimentally determined for blood, fat, liver, kidney, muscle, brain, and lung tissues using vial equilibration. Metabolism of TMP was assumed to occur in liver and kidney. Initial model simulations depicting temporal levels of TMP in the gas uptake chamber closely resembled data. The availability of a calibrated PBPK model for TMP is useful in establishing dose-response relationship between in vivo brain levels of the chemical and ensuing neurotoxicity measures. (This abstract does not reflect EPA policy.)

1004 PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELING OF P-TERT-OCTYLPHENOL IN MALE AND FEMALE SPRAGUE-DAWLEY RATS.

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This study was undertaken to characterize the toxicokinetics of p-tert-octylphenol (OP) in rats and to develop a physiologically-based pharmacokinetic (PBPK) model. Groups of 5 male and 5 female Sprague-Dawley rats were given a single dose of OP by oral (*po*) (50, 125 or 250 mg/kg), intravenous (*iv*) (2, 4 or 8 mg/kg) or subcutaneous (*sc*) (125 mg/kg) routes. Blood samples were collected up to 4 h (*iv*) and 24 h (*po* and *sc*) post-exposure. A satellite group of animals receiving the same treatment was sacrificed after 1 and 3 h (*iv*) or 4 and 24 h (*po*) post-exposure and tissues (fat, liver, testes and uterus) were analysed for OP content by GC-MS. A PBPK model was developed to simulate blood and tissue OP concentrations in rats following *po*, *sc* and *iv* exposure. Compartments for the model included the liver, richly perfused tissues, poorly perfused tissues, reproductive tissues, adipose tissue and skin. Metabolism was described to occur primarily in the liver. Binding as well as solubility were considered for computing partition coefficients. The model predictions were consistent with rapid decrease of blood OP concentrations for all exposure routes. The half-life of OP in rats was calculated to be 1.1 hr (male) and 1.3 hr (female). A marked sex-difference was seen in blood and tissue OP concentrations. Bioavailability of OP for the *po* and *sc* routes were higher in female than in male rats. Oral bioavailability ranged from 28 to 36% (male) and from 46 to 55% (female) whereas *sc* bioavailability was 29% (male) and 44% (female). A significant difference was also observed in the metabolism of OP between male and female rats, which was reflected in the model. Overall, the PBPK model successfully simulated blood and tissue concentrations of OP in Sprague-Dawley rats following administration by several routes. Further studies are required to elucidate the mechanism underlying the gender differences observed in this study and to evaluate whether that is also observed in humans. (Supported by NSERC, Canada).

1005 PBPK MODELING OF CHLORZOXAZONE AND ITS 6-HYDROXY METABOLITE IN HUMANS.

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Physiologically-based pharmacokinetic (PBPK) models have not been developed for many substrates used as probes of CYP isozymes. Chlorzoxazone (CZX), a central muscle relaxant has been proposed as a non-invasive probe of in vivo CYP2E1 activity. The objective of this study was to develop a PBPK model for simulating the plasma concentrations of CZX and its 6-hydroxy metabolite (HCZX) in humans. A four-compartmental model for CZX (liver, fat, gastro-intestinal tract and rest of body) was linked with a single compartmental description for HCZX. The input for the CZX model was oral dose whereas the input for the HCZX model was the rate of metabolism of the parent chemical. The parameters required for solving the equations constituting the integrated CZX-HCZX model were: physiological parameters, volume of distribution (HCZX) or partition coefficients (CZX) as well as clearance parameters. Physiological data (cardiac output, tissue plasma flows and tissue volumes) were obtained from the literature. Biochemical parameters, Michaelis-Menten affinity constant (Km) and maximal metabolic velocity (Vmax) were obtained from a previously published in vitro study. Vmax was computed on the basis of information on the enzyme turnover number and hepatic content of CYP2E1. The tissue:plasma partition coefficients (Ptp) required for the CZX PBPK model were calculated as a function of interstitial fluid content; the estimated Ptp was 0.1 for fat and 0.2 for other tissues. The half-life (1.45 hr) and renal clearance (25.8 L/hr) data for HCZX were used to estimate its volume of distribution (54 L). The resulting PBPK model provided adequate simulations of previously published data on the plasma concentrations of CZX and HCZX, as well as the metabolic ratio (HCZX/CZX) measured at 2, 4 and 6 hr in humans receiving 500 mg CZX (po). This model should be useful for evaluating the relationships among HCZX/CZX ratio, time of measurement, hepatic levels of CYP2E1 and human physiology.

1006 ASSESSMENT OF DICHLOROMETHANE PBTK MODEL PERFORMANCE IN THE RAT.

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Recent advancements in physiologically based toxicokinetic (PBTK) models for dichloromethane (DCM) include a Bayesian-calibrated model for the mouse. The mouse is the more sensitive animal species with respect to cancer risks, but rats appear to be more sensitive to noncancer effects from DCM than mice. We evaluated rat models for predicting DCM internal doses relevant to noncancer endpoints (liver lesions) in rats; these doses could serve as the basis of a RfD or RfC for an IRIS assessment. Data for blood DCM and carboxyhemoglobin, exhaled DCM, and gas uptake from iv and inhalation exposures were collected from the literature. Five models were evaluated for their ability to replicate these data, predict the ratio of CYP:GST metabolites produced in the liver, and predict total liver CYP and GST metabolites. The five models were: 1) an allometrically scaled version of the Bayesian-calibrated mouse model; 2) an adaptation of the Bayesian-calibrated mouse model with rat-specific model parameter values; 3) a published rat model that simulates carbon monoxide (CO) generation; 4) model 3 modified to include lung metabolism (with parameters derived from in vitro data); and 5) model 4 with re-optimized metabolic parameters. Model 1) did not adequately predict DCM and CO levels. Models 2-5 gave similar, adequate predictions of DCM and CO levels with iv and inhalation exposures. However, models 3-5 gave better predictions of

the gas uptake data than model 2. Comparison of models 3 and 4 (rat model with and without lung metabolism) yielded similar predictions of total metabolite production or CYP:GST production ratio. The inclusion of lung metabolism in model 4 makes it more biologically realistic. These comparisons suggest that model 4 is appropriate for use in deriving the RfD and RfC for DCM. Views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA.

1007 SEQUENTIAL BAYESIAN ANALYSIS OF THE FORMALDEHYDE CANCER MODEL, PART 2: CALIBRATION OF RAT CLONAL GROWTH MODEL.

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A sequential Bayesian approach is being conducted to provide a comprehensive quantitative uncertainty evaluation of the formaldehyde clonal growth model (Conolly et al, 2003). The first step, a Markov Chain Monte Carlo (MCMC) analysis for the sub-model that predicts formaldehyde-induced DNA-protein crosslink (DPX) formation, has been reported previously. In the current study, MCMC calibration of the 2-stage clonal growth model in the rat was performed using time-to-tumor data from F344 rats exposed to formaldehyde at concentrations up to 15 ppm (Monticello et al. 1996). The DPX predictions generated in the previous MCMC analysis were used as inputs to clonal growth model in this analysis. The cancer model also includes several formaldehyde-independent parameters related to the normal processes of cell proliferation and mutation that result in the background occurrence of nasal tumors. Although the observed response rate in the concurrent controls in the Monticello bioassay is zero, the Bayesian approach makes it possible to use the historical record of nasal squamous cell carcinoma (SCC) in F344 rats as prior information to suggest that the background response rate cannot be zero. Rather than using historical control data to "fit" the 2-stage model, as was done previously, the information derived from (1) all or (2) inhalation-only NTP historical controls was used to define priors for the parameters responsible for background response patterns. This approach allowed background tumor rates to be considered without constraining the model to treat the historical controls as if they were concurrent controls. In the next phase of this study, posterior parameter distributions from the current analysis will be used to inform priors for the human clonal growth model.

1008 A QUANTITATIVE MODEL FOR XENOBIOTIC METABOLIZING ENZYME (XME) INDUCTION REGULATED BY THE PREGNANE X RECEPTOR (PXR).

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The nuclear receptor, PXR, is an integral part of the regulation of hepatic metabolism. It has been shown to regulate specific CYPs (phase I drug-metabolizing enzymes) as well as certain phase II drug metabolism activities, including UDP-glucuronosyl transferase (UGT), Sulfotransferase (SULT), and glutathione S-transferase (GST). With its large and flexible ligand-binding domain, PXR can be activated by a broad range of relatively small, hydrophobic exogenous compounds. Upon activation, PXR partners with the Retinoid X Receptor (RXR) to form a heterodimer. The newly formed heterodimer binds to an appropriate DNA response element, causing increased transcription. This leads to an induction in the level of XMEs. These mechanistic steps are included in a
biologically-based mathematical model. The quantitative model predicts fold induction of mRNA and protein of CYP3A4 in response to PXR activation. Initial sensitivity analysis of the model suggests that CYP3A4 levels are most affected by the transcription rate, translation rate, and CYP3A4 degradation rate. Parameter values were either based on information reported in the literature or optimized to available in vitro data. Model predictions are compared to literature data of CYP3A4 mRNA and protein levels in response to various doses of rifampicin, a known PXR activator. This initial modeling effort will be expanded to include other relevant nuclear receptors. Further calibration and evaluation of the current and expanded model will be performed using
computational tools and in vitro and in vivo experiments utilizing different environmentally relevant chemicals. (This abstract does not reflect EPA policy)

1009 ASSESSING FACTORS IMPORTANT FOR TISSUE ACCUMULATION OF MANGANESE IN MONKEYS BY PHARMACOKINETIC MODELING.

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Manganese (Mn) is an essential nutrient required for normal tissue growth and function. In addition to intake from food, water and air, exposure may also arise from low concentration of Mn particulates produced in certain occupations or from combustion of Mn-containing fuel additives. Following exposures to high concentrations of inhaled Mn, there is preferential accumulation of Mn in certain brain regions such as striatum and globus pallidus, despite the fact that tissue Mn concentrations are tightly regulated to prevent toxic accumulation. The purpose of our research was to extend a rodent physiologically-based pharmacokinetic (PBPK) model for Mn to describe tissue accumulation of this metal in primates exposed to Mn by inhalation and diet. Model parameters were first calibrated using steady state tissue Mn concentration in rhesus monkeys fed on a diet containing about 130 ppm Mn. The model was then applied to evaluate 15, 33 or 65 days of repeated (6 h/day; 5 days/week) inhalation exposure data of soluble $MnSO_4$ at 0.0, 0.06, 0.3 or 1.5 mg Mn/m^3. Sensitivity analysis showed that Mn tissue concentrations in the monkey were affected by (1) dose-dependent biliary excretion of free Mn from liver, (2) saturable tissue binding in all tissues, and (3) differential influx/efflux rates for those brain regions that accumulate Mn. This multi-species PBPK model is consistent with the available experimental kinetic data, indicating preferential increases in some brain regions with exposures above 0.2 mg/m^3 and rapid return to steady state levels after cessation of exposure. These PBPK models which include preferential tissue accumulation for both oral and inhaled exposures provide support for tissue dosimetry based risk assessments for manganese, an essential dietary nutrient with high dose toxicity.

1010 UNDERSTANDING THE COMPLEXITY IN KINETICS OF PERFLUOROOCTYLACIDS IN MONKEYS AND RATS USING A PHARMACOKINETIC MODEL.

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Perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS) have elimination half-lives in human serum of several years; but elimination half-lives in laboratory animals are noticeably shorter: months for monkeys and days for rats. In order to identify the biological determinants of reported species and gender differences in elimination of these compounds, a multi-compartment pharmacokinetic model (liver, deep tissue, filtrate, and central compartments) was developed. The model includes two time-dependent processes: free fraction in plasma decreases with time, while volume of distribution increases with time. A similar model structure was used for PFOA and PFOS in both cynomolgus monkeys and rats. The model was able to reproduce the complex time-course of plasma concentrations in male and female monkeys exposed intravenously to PFOS, as well as plasma, urine and feces time-courses in rats exposed to PFOS and PFOA. The use of a structurally similar pharmacokinetic model allowed examination of the kinetics observed in animal studies by comparing the calibrated model parameters. A higher estimated liver:blood partition coefficient in the rat, as well as the need for additional binding in rat liver suggest that PFOS retention in liver occurs in male rats but not in male monkeys. On the other hand, the renal transport maximum for monkeys was about 1500 times higher than that for rats. Comparison of model parameters for PFOS and PFOA suggests that PFOS is retained longer in tissues compared to PFOA, as indicated by a higher liver:blood partition coefficient, a higher renal filtration, and a much smaller rate constant from the deep tissue compartment to the central compartment. Comparison of PFOA parameters for male and female rats suggests that the slower renal clearance in male rats exposed to PFOA could be a result of lower levels of key organic anion transporters such as OAT2. Understanding these cross-species, cross-gender, and cross-compound difference is an important step in the development of a human model for these compounds.

1011 ENHANCING THE MODELING OF PFOA PHARMACOKINETICS WITH BAYESIAN ANALYSIS.

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The detail sufficient to describe the pharmacokinetics (PK) for perfluorooctanoic acid (PFOA) and the methods necessary to combine information from multiple data sets are both subjects of ongoing investigation. Bayesian analysis provides tools to accommodate these goals. We examined the results of a series of experiments [Kemper, 2003] that observed the concentration of PFOA in male and female adult rats. For some subjects plasma concentration was observed over time, while for others feces and urine were collected. Assuming that the PK parameters for each individual were drawn from the same, biologically-varying population, we jointly analyzed both types of data. This hierarchical approach allowed us to estimate the contributions of uncertainty due to measurement error and actual biological variability.

We performed a Bayesian analysis using Markov Chain Monte Carlo. Since PFOA excretion is known to be sexually dimorphic in rats, we analyzed the male and female data separately. The distributions of integrated plasma concentration (AUC) varied significantly because the clearance is much higher in females (35.6±9.2 mL/kg/h) than in males (0.81±0.24 mL/kg/h).

Starting from a one-compartment PK model with separate clearances to urine and feces, we incrementally expanded the model using Bayesian measures to assess if the expansion was supported by the data. Including excretion data initially decreased certainty in the AUC compared to an analysis using plasma data only. Allowing a third, unspecified clearance improved agreement and increased certainty when all the data was used. However, the PFOA cleared by this third route appeared too large to be experimentally plausible. Adding an additional PK compartment was supported by the data and reduced the unaccounted-for elimination to amounts comparable to the cage wash. Since we have established that the data require more complicated models, PBPK models may provide additional insights.
This work was reviewed by EPA and approved for publication but does not necessarily reflect official Agency policy.

1012 MODELING THE PHARMACOKINETICS OF PERFLUOROOCTANOIC ACID DURING GESTATION AND LACTATION IN MICE.

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Perfluorooctanoic acid (PFOA) is used as a processing aid for the production of commercially valuable fluoropolymers and fluoroelastomers. It has been widely detected in biological organisms including humans whose estimated blood levels are in the low ppb levels for the general US population. PFOA is metabolically stable and exhibits a plasma half-life of 3-5 years in humans. In mice, PFOA induces developmental toxicity in the form of full litter resorption, compromised postnatal survival, delayed growth & development, and altered pubertal maturation. While some postnatally observed developmental effects have been attributed to gestational exposure, it remains to be elucidated whether these result from a higher internal dose (pharmacokinetics) and/or exposure during a developmentally sensitive period (pharmacodynamics). To address the pharmacokinetics of PFOA during gestation and lactation, a biologically-supported dynamic model was developed. A two compartment system linked via placental blood flow described gestation, while milk production linked the dam to a pup litter compartment during lactation. Mathematical functions described the growth of the dam, conceptus, placental blood flow, and nursing pups. Serum:fetal and serum:milk partition coefficients and milk production were estimated from published literature. Absorption and elimination were described as 1st order processes. The model reasonably simulated reported serum levels for non-lactating dams at doses equal or less than 1 mg/kg PFOA, but failed to simulate non-linear behaviors at higher doses. A reduction in the milk elimination rate constant, probably reflecting data gaps in model calibration, was necessary to simulate levels in lactating dams and pups at weaning. Conceptus levels start low relative to maternal serum but increase rapidly towards the end of gestation as a consequence of the estimated partitioning. These modeling efforts provide an initial template for further explorations of the pharmacokinetics of PFOA relevant to one-generation toxicity studies. (This work does not reflect official Agency policy).

1013 A BIOLOGICALLY BASED DOSE-RESPONSE MODEL FOR DIETARY IODIDE AND THE ADULT RAT THYROID AXIS: APPLICATION TO IODIDE DEFICIENCY AND PERCHLORATE EXPOSURE.

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A biologically based dose-response (BBDR) model for dietary iodide and the adult male rat hypothalamic-pituitary-thyroid (HPT) axis (BBDR-HPT model) was developed and used to describe perturbations in the HPT axis caused by iodide deficiency and perchlorate. The BBDR-HPT model includes sub-models for dietary iodide, thyroxine (T_4), 3,5,3'-triiodothyronine (T_3), and thyroid stimulating hormone (TSH) that are linked via biological processes such as the T_4/TSH negative feedback, TSH stimulation of thyroidal processes, and recycling of iodide from metabolism of thyroid hormones. The first application of the calibrated BBDR-HPT model was to test its ability to predict changes in serum TSH, T_4, T_3, and total thyroid iodide stores resulting from an insufficient intake of iodide. Model behavior was consistent with the perturbations (serum TSH, T_4, T_3, and thyroid iodide) in the HPT axis under iodide deficient conditions. Several environmental chemicals (e.g. perchlorate and thiocyanate) inhibit the uptake of thyroidal iodide, resulting in decreased production of thyroid hormones, presumably by decreasing available thyroidal iodide. Our BBDR-HPT axis model was tested for its ability to predict changes in the HPT axis when linked with a physiologically based pharmacokinetic (PBPK) model for perchlorate. When nothing more than competitive inhibition of thyroidal iodide uptake by perchlorate was included, the linked models were not able to capture the rapid decline in serum T_4 and increase in serum TSH responses when laboratory rats were exposed to perchlorate in drinking water. Perchlorate appears not only to affect iodide availability for thyroid hormone production, but also to interfere with production of thyroid hormones, a process that gradually recovers under the influence of TSH. (EPA STAR Fellowship #FP916793 and Grant #RD832134)

1014 PREDICTING LACTATIONAL AND EARLY POST-WEANING EXPOSURES IN RATS USING BIOLOGICALLY BASED PHARMACOKINETIC MODELING.

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Risk and safety assessments for early life exposures to environmental chemicals or pharmaceuticals based on cross-species extrapolation would greatly benefit from information on chemical dosimetry in the young. Although multiple life stage exposures are involved, maternal dose is frequently used to extrapolate rodent toxicity findings to humans representing a substantial source of uncertainty. A compartmental pharmacokinetic model augmented with biological factors changing during lactation and early post-weaning was developed. The model uses adult pharmacokinetics, milk distribution, and relevant postnatal biology (e.g., dam and pup body weights, milk and diet consumption) to predict dosimetry in the young for chemicals with a range of characteristics. The model predicted pup exposure to a reasonable approximation as shown by comparison with published data for two chemicals. It enables simultaneous consideration of multiple factors that affect pup exposures, providing a means to predict the overall impact of these factors. Factors addressed in the model include three dosing strategies employed in toxicity studies (gavage, adjusted and unadjusted diet), pharmacokinetic properties including rates of clearance, milk distribution, and volume of distribution, developmental delays in clearance and recirculation of chemical in excreta from pup to mother. It was demonstrated that pup exposures do not always parallel the mother's. Pup exposure was generally lower than the mother's for theoretical chemicals with a shorter half life, lower milk transfer, larger volume of distribution, and gavage dosing, while higher with longer half life, higher milk transfer, smaller volume of distribution, and dietary exposures. The model predictions can be used to help design early life toxicity and pharmacokinetic studies and better interpret study findings, leading to improved risk assessments (EPA-NRC #CR82879001. This abstract may not represent official EPA policy).

1015 UTILIZING PK/PD RELATIONSHIPS FOR INHIBITION OF SOD1 EXPRESSION IN BRAIN AND SPINAL CORD (CNS) OF RATS AND MONKEYS TO PREDICT HUMAN ASO DOSE.

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Studies were conducted to characterize the effect of SOD1 antisense inhibition on SOD1 mRNA expression together with the absorption, distribution and elimination of the antisense molecule in the CNS of rats and monkeys. A potent inhibitor of SOD1 expression, with cross-species specificity to a conserved region of the SOD1 mRNA, was identified (ISIS 333611). ISIS 333611 is a phosphorothioate 20-mer oligonucleotide partially modified with 2′-O-methoxyethyl nucleotides at the 3′ and 5′ termini of the oligonucleotide. We have explored intrathecal (IT) and intracerebroventricular (ICV) administration. This compound produced dose and concentration-dependent reduction of SOD1 mRNA in brain and spinal cord of rats. Effective brain and spinal cord drug concentrations were identified as 4 to 8 μM (approximately 30 to 60 μg/g). These levels were achieved at doses of 100 μg/day continuous infusion in rats and 300 μg/day continuous infusions in monkeys administered for 2 or 4 weeks. The antisense compound produced a long duration of mRNA reduction consistent with the long half-life (>1 month) determined in rat brain and spinal cord. Pharmacokinetic models have been developed that include species-specific variables for cerebrospinal fluid (CSF) turnover, size of brain (distribution volume in the CNS), and half-life of clearance from CNS tissue that provide reasonable prediction from mouse to monkey. Thirteen week toxicology studies conducted in rats and monkeys were used to validate the models for longer term continuous infusion. Human doses are proposed based on the PK model together with active target tissue concentrations in the nonclinical models.

1016 PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELING OF AN ALKYLBENZENE MIXTURE IN CHILDREN OF VARIOUS AGE GROUPS.

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A physiologically based pharmacokinetic (PBPK) model describing metabolic interactions among toluene (T), m-xylene (X) and ethylbenzene (E) has previously been developed and validated for adult male volunteers. The objectives of the present study were to adapt this model to predict metabolic interactions in children and to estimate adult to children variability in internal doses during mixed exposures. The approach consisted of using age-specific values of (i) physiological parameters (cardiac output, alveolar ventilation rate, tissue blood flows and volumes), and (ii) liver content of CYP2E1, in the PBPK model. For different stages of child development, PBPK model simulations of inhalation exposure to a TEX mixture (1/3 TLV of each substance; T 17 ppm+ X 33 ppm + E 33 ppm) were generated. Following a sensitivity analysis, distributions of key parameters of the internal dose of TEX (alveolar ventilation rate, CYP2E1 content and cardiac output to alveolar ventilation rate ratio) were integrated into the models. Monte-Carlo simulations suggested that only neonates have a potential of being internally exposed to higher levels of TEX compared to adults. The 95th percentile of venous blood concentrations simulated for the adult and neonate populations were respectively 0.51 and 0.69 mg/L. The interindividual variability in internal dose of TEX in adults and neonates was such that no significant difference can be detected between the two sub-populations. The average adult to neonate factor, however, was lower during TEX mixture exposures (2.17) than during single compound exposures (2.64). This study demonstrates the feasibility of modeling metabolic interactions in children as well as the importance of interindividual variability within sub-populations in evaluating internal dose during mixed exposures.

1017 PBPK MODELS FOR MIXTURES OF DIOXIN-LIKE CHEMICALS.

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The National Toxicology Program (NTP) conducted multiple 2–year rodent bioassays in female Harlan Sprague Dawley rats examining the carcinogenicity of TCDD, PeCDF, PCB126, PCB153, and PCB118 as well mixtures of these chemicals. A physiologically based pharmacokinetic (PBPK) model for TCDD was used as the framework to model the individual chemicals and the mixtures. A goal was to assess how a model developed specifically for TCDD would serve as a general Ah receptor model for individual dioxin-like chemicals as well as a mixture of dioxin-like chemicals. The mixture model links the individual chemicals with competitive binding to the Ah receptor and CYP1A2. The NTP bioassay data includes lung, liver, blood and fat concentrations as well as EROD and A4H activity. The individual chemical models were fit to the NTP data. The parameters from the individual models were used in the mixture models to make predictions to compare with mixture data. The modeling accounted for the possible differences in binding, metabolism, cytotoxicity, and absorption of each individual chemical. We used likelihood ratio tests to evaluate if various groups of model parameters provide superior model fits. Accurate model predictions are possible for the other dioxin-like chemicals when only changing a small number of the original TCDD model parameters. The models make good predictions of the tissue concentrations as well as the P450 activity levels. The models are part of an ongoing effort to develop a general Ah receptor PBPK model.

1018 EFFECT OF DYNAMIC BLOOD PARTITIONING DURING LATE GESTATION ON CHLORPYRIFOS DOSIMETRY.

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Chlorpyrifos and its active metabolite, chlorpyrifos-oxon, are lipophilic organophosphorous pesticides. Blood lipids increase during pregnancy, and varying maternal lipid profiles may be associated with different birth outcomes. Epidemiological studies have recently evaluated blood chlorpyrifos levels near parturition and are attempting to correlate those levels to birth outcomes. However, relating blood chlorpyrifos levels to birth outcomes without accounting for blood lipid changes is likely to be misleading. The blood:propylene carbonate partitioning of chlorpyrifos in rat blood was dynamic over the course of late gestation and partitioning was ~10x greater in GD18 than non-pregnant rats. A chlorpyrifos PBPK/PD was modified to accommodate physiological and dynamic changes of blood partitioning. Specifically, an equation describing the dynamic changes in blood:propylene carbonate partitioning was used to estimate tissue:blood partitioning of chlorpyrifos and chlorpyrifos-oxon from GD16-20. The model describes the available data well and demonstrates that increases in blood solubility during late gestation results in peak blood chlorpyrifos levels that are 3 to 15x higher in pregnant than in non-pregnant rats at chlorpyrifos doses in the 1 mg/kg range. Published rodent data indicate the elevated blood concentrations during pregnancy have little or no impact on clinical signs. The rodent model was extrapolated to humans to evaluate the impact of the dynamic blood solubility on the dosimetry of chlorpyrifos in pregnant women. For the same level of environmental exposure, the model predicts the blood chlorpyrifos AUC in pregnant women will be ~10X higher than in a non-pregnant adults. Using the low pg/g plasma CPF levels found in women at childbirth in the epidemiological studies, the PBPK model estimated an environmental exposures of ~40 ng/kg body weight per day. This model- predicted dose is similar to estimates from other recent publications for children and adults.

1019 A DISCRETE TIME MODEL TO ANALYZE THE EFFECTS OF TOXICANTS ON THE GROWTH OF C. ELEGANS NEMATODES IN A MEDIUM THROUGHPUT SETTING.

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The National Toxicology Program is developing methodologies for the analysis of high- and medium-throughput screening data to determine the toxicity of compounds. In that context, we present a stochastic model for the analysis of growth and development of the nematode C. elegans. The model uses flow cytometry (COPAS Biosort) data consisting of optical extinction (EXT) and time of flight (TOF) measurements of individual nematodes as they are loaded into 96 well plates before exposure. The nematodes are measured, by aspiration into the Biosort, after 12, 24, 36, 48, 60, or 72 hours. A Markov model is presented that describes growth with respect to log(EXT) and log(TOF) measurements between aspiration times. The model uses the observed distribution of measurements at loading to predict the distributions at the aspiration times. By including a distribution for noise in the cost function, the model allows the analysis of measurements made on living nematodes unaffected by measurements made on detritus. Optimizing the same basic model with minor modifications allows estimates of different physiological characteristics when applied to EXT and TOF measurements separately. When applied to EXT measurements, the model supplies estimates of the number of living nematodes that are aspirated at each time point, as well as growth rates with respect to EXT units. When applied to TOF data, the model allows estimates of the growth rates with respect to TOF units as well as the percent of nematodes curling as they travel through the flow tube, and the attenuation fraction caused by the curling. For illustration, the model is applied to growth data from unexposed C. elegans.

1020 THE DEVELOPMENT OF A MULTISCALE CARDIAC MODEL OF NANOPARTICLE DRIVEN ATHEROSCLEROSIS.

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There is growing evidence that acute exposure to ambient particulate matter may be involved in lesions of the coronary endothelium that lead to its onset. At the same time, it is well known that deleterious alterations in the fluid shear stress and coronary transmural pressure perturb endothelial biochemistry and exacerbate the local inflammation at the root of atherosclerotic plaque formation. At PNNL we have initiated a program to develop a multiscale computational model of the heart to investigate the relationship among atherosclerosis, the presence of nanoparticles and the secondary perturbations of respiratory inflammation. The project aims to link cardiovascular function with respiratory function and biomechanics with biochemistry. A preliminary mouse heart geometry with cellular resolution was reconstructed from serial cryomicrotome images via a nonlinear constrained elasticity approach. In addition, a high-contrast, high resolution, whole mouse, contrast-infused magnetic resonance (MR), dataset was acquired. A detailed segmentation of both tissue and blood domains was performed with custom algorithms including fuzzy connectivity, and a network preserving algorithm for extracting the coronary vasculature. To create a computational mesh, tissue surfaces, blood surfaces and blood-tissue interface were extracted with a multi-material

marching cubes algorithm. This was followed by application of a novel scale-invariant, multi-material mesh-generation algorithm that produces a quality, nearly orthogonal paving of biological geometries based on the concept of local scale. Finally, we have developed efficient constitutive models for myocardium, papillary muscle, chordae tendinae, cardiac valve tissue and coronary artery. This work is leading towards a predictive computational model linking circulation to respiration, which can become an important tool for predicting regionally specific deposition of ultrafine particulates and site selectivity of atherosclerotic plaque formation.
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1021 DIMENSIONAL ANALYSIS TO PREDICT THE SPECIFIC ISOFORMS INVOLVED IN BINDING CYP SUBSTRATES.

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Few enzymes are more striking in both versatility and in sheer number of substrates than the cytochrome P450 (CYP) enzyme system. The metabolism of environmental pollutants and drugs in humans may be mediated by one or more of over fifteen different CYP isozymes. The ability to classify these xenobiotics according to the CYP(s) that catalyze their biotransformation has numerous implications for toxicology. For instance, such a capability may facilitate the understanding of important pharmacokinetic interactions that affect the toxicity of these substances, especially for chemical mixtures.
Here we present a novel method of classification and clustering based on a dimensional analysis of a broad range of substrate chemical descriptors. Although dimensional analysis has been widely used in the physical sciences for decades, it has received little attention in the biological sciences. In the present work, constitutional, geometric, physicochemical, electronic, and energetic descriptors of a diverse training set of substrates were combined into a set of optimally-descriptive dimensionless groups based on their ability to cluster substrates of CYP1A1, CYP1A2, CYP2C9, CYP2E1, CYP2D6, and CYP3A4. These dimensionless groups were then used to create a set of logistic equations that provided good clustering predictions for a large set of validation compounds. This general methodology may be readily enhanced by incorporating new molecular descriptors as they are identified in the future.
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1022 AN UPDATED METHODOLOGY TO PREDICT RATES OF CYTOCHROME P450 MEDIATED HYDROXYLATION OF ALIPHATIC SUBSTRATES.

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The cytochrome P450 (CYP) enzymes mediate phase I biotransformations, primarily acting as a monooxygenase, where an oxygen atom is incorporated into substrates. These reactions usually convert hydrophobic xenobiotics into polar forms, making them more water soluble and thus more readily excreted. Unfortunately, this process can also activate certain substrates, such as potential carcinogens, via transformation into electrophilic species capable of interacting irreversibly with biological molecules. The rates of CYP-mediated metabolism influence the importance of various biotransformation pathways, affect the abundance of certain potentially-toxic metabolites, and alter the clearance characteristics of the chemical species. Thus, the ability to predict these rates is crucial in assessing the risks posed by xenobiotics. Here we present a computational model for the prediction of substrate hydroxylation via hydrogen abstraction mediated by CYPs. This model improves and extends the pioneering work of Korzekwa et al. (*J. Am. Chem. Soc.*, 112, 1042-1046, 1990) and Yin et al. (*Proc. Natl. Acad. Sci.*, 92, 11076-11080, 1995). The model is based on the use of a surrogate model for the heme-iron oxygen species of the CYPs: *p*-nitrosophenoxy radical (PNR). The semi-empirical quantum chemical method, PM3, was used to compute the PNR-substrate transition state, along with the optimal conformations and energetics of the participating species. These results were then used to create a linear free energy-based equation relating the activation energy to the heat of reaction and the ionization potential of the intermediate carbon-centered radical. Predictions from this model equation were found to be in excellent agreement with the results described in Korzekwa et al. (*J. Am. Chem. Soc.*, 112, 1042-1046, 1990).
This study was supported, in part, by NIEHS grants K25 ES11146 and K25 ES012909.

1023 ANALYSIS OF DERMAL PENETRATION OF TITANIUM DIOXIDE (TIO2) FROM SUNSCREEN FORMULATIONS CONTAINING MICRON- AND NANO-SCALE PARTICLES OF TIO2.

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Nanoscale materials are being incorporated into a variety of commercial products, including topically applied sunscreens. In order to help address certain concerns regarding dermal penetration of nanoscale materials in these products, a study was conducted to assess whether nano-scale TiO2 contained could penetrate skin to a greater extent than micron-sized TiO2 in the same vehicle. The study was conducted in female Yucatan minipigs because pig skin is structurally most similar to human skin. Identical sunscreen base formulations were made containing no TiO2, 5% micron-sized TiO2, 5% nanometer-sized, uncoated TiO2 or 5% nanometer-sized, Aluminum hydroxide and dimethicone/methicone copolymer coated TiO2. These formulations were dermally applied to pigs at a dose of 2 mg emulsion/cm2 skin for 5 days per week for a period of 4 weeks. At the end of the treatment, the animals were euthanized and the following tissues were removed using titanium-free necropsy methods: skin (multiple sites), lymph nodes, liver, brain, spleen, kidneys. The presence of TiO2 in the tissues was monitored through ICP-MS (inductively coupled plasma mass spectroscopy) analysis of the titanium following digestion of tissue samples. Our results to date indicate that there is no increase in the level of Ti in lymph nodes and liver of any of the treated animals, over the baseline levels of Ti. This suggest no systemic distribution the TIO2 applied skin. All epidermal skin samples showed elevated levels for all sunscreens containing TiO2, whereas in the dermis Ti levels were elevated in some of the TiO2-treated groups. Skin samples are currently being analyzed using SEM-EDX (scanning electron microscopy-energy dispersive X-ray analysis) to determine whether there may be penetration of TiO2 at the junction of the dermis and epidermis or whether there may be accumulation of TiO2 in certain structures, such as hair follicles.

1024 THE TOXICITY AND BIOCOMPATIBILITY OF NANOMATERIALS IN HUMAN KERATINOCYTES (HACAT CELLS).

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Currently, many companies are incorporating nanomaterials into their products and consumers are coming into contact with nanomaterials that can be absorbed through the skin or ingested/inhaled into the body. This study was designed to determine the biocompatibility or toxicity of various nanoparticles using human keratinocytes (HaCaT cell line) as a model. We evaluated the cytotoxicity of gold (Au) nanoparticles with different ligands (MEEE which makes the particles neutral, MES which makes the particles anionic, and TMAT which makes the particles cationic) with 0.8nm, 1.5nm and 10nm diameters, and silver (Ag) 15nm particles coated with lysozyme. CytoViva microscopy was used to visualize the uptake of 10 μg/ml of nanoparticles into the keratinocytes. MTS, LDH and ROS assays were performed to assess the toxicity of the nanoparticles at different concentrations (0, 10, 25, 50, and 100 μg/ml). CytoViva showed that all the nanoparticles were taken up into the cells. In addition, the results of the toxicity tests demonstrated that the silver particles were non-toxic, but the 0.8nm and 1.5nm gold particles regardless of ligand were toxic in a concentration dependent manner, and showed a significant decrease in cell proliferation and substantial membrane leakage. However, the 10nm TMAT gold particles were non-toxic while the 10nm MES gold particles were toxic in a concentration dependent manner. Future studies with the silver and non-toxic gold nanoparticles will test their anti-microbial properties in bacteria.

1025 THE ROLE OF SIZE IN TITANIUM DIOXIDE DERMAL NANOTOXICITY.

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Currently there is conflicting data on what mediates the toxicity of titanium dioxide (TiO_2) nanoparticles. In this study, we wanted to evaluate the cytotoxic effect of varying sizes of TiO_2 nanoparticles with no variation in crystral structure to see if there was a correlation between nanosize and toxicity. The human keratinocyte cell line, HaCaT, was used as a model, and the sizes of TiO_2 nanoparticles were 6.3, 10,

50, 100, 194 nm and the crystal structure was 100% anatase. Dynamic light scattering (DLS) was performed to assess the degree of agglomeration once the particles were in solution. Furthermore, MTS and LDH assays were performed at a 24 h timepoint to assess for disruption in cell proliferation and membrane leakage, respectively at varying concentrations (0-150 μg/ml). ROS data was evaluated after 24 h to check for oxidative stress. Cytoviva microscope analysis was also performed to visualize the uptake of the TiO_2 nanoparticles in keratinocytes. The DLS studies determined that the nanoparticles agglomerate in water and media and that serum decreased the degree of agglomeration. The MTS assay showed that there was not a significant decrease in cell proliferation after 24 hours for the larger particles. However, the 6.3 nm TiO2 showed a significant decrease in proliferation at higher concentrations (> 100 μg/ml). Similarly, at 24 h found only the 6.3 nm particles showed a significant increase in membrane leakage. Using the ROS assay, at a concentration of 10 μg/ml there was no evidence of oxidative stress for all the particles tested. However, at the 100 μg/ml concentration, the particles that were > 50 nm, showed a significant increase oxidative stress. Furthermore, the Cytoviva imaging showed that particles are taken up by the cells. Further studies will be performed to compare the size dependent toxicity data with crystal structure dependent data.

1026 STUDIES ON SIO_2 NANOPARTICLE CHARACTERIZATION AND ITS RELATIONSHIP TO TOXICITY IN KERATINOCYTES.

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The objective of this study was to demonstrate the relationship of silicon dioxide (SiO_2) nanoparticle characterization (size, and agglomeration) and toxicity in mouse keratinocytes (HEL-30) and human keratinocytes (HaCaT) by examining cell uptake and changes in viability. To study toxicity, HaCaT cells were dosed with various sizes of SiO_2 nanoparticles (30, 51, 110 and 400nm) at different concentrations (0, 10, 50, 100 and 200μg/mL) for 24h. Size- and dose-dependent MTT reduction was observed. Except for the largest size studied (400nm), SiO_2 nanoparticles (35, 51 and 110 nm) at 100 and 200μg/mL showed significant reduction of 3-15% of control. In comparison, the HaCaT cells were more sensitive to SiO_2 nanoparticles than HEL-30 cells. In addition, various sizes of SiO_2 nanoparticles (35, 51, 110 and 400nm) at 50μg/mL were suspended in water and in exposure media to study agglomeration by DLS method at cold (4°C) and warm (37°C) conditions. Time points were 0, 8, 14, 22, 32 and 48h after preparation of samples. There were no size changes observed in all of nanoparticles in both temperatures except 35 and 51 nm SiO_2 particles in exposure media at 37°C. Sizes of 35nm SiO_2 were increased to 3800nm at 14h time point; gradually decreased to 400nm at 48h. For 51nm SiO_2, agglomeration reached to 2200nm at 14h, then down to 500nm at 22h; increased to 1500nm at 32h and decreased to 500nm at 48h. This experiment provides the information that the sizes changed during the 24h exposure of SiO_2 nanoparticles at 37°C. To verify the uptake of nanoparticles, HEL-30 cells were incubated with 100μg/mL of SiO_2 for 24h, then imaged with a TEM. Cells showed internalization of all of the different sizes of SiO_2 nanoparticles into cytoplasm. Based on the results, SiO_2 nanoparticles show size- and dose-dependent toxicity in HaCaT, and size-dependent agglomeration in exposure media at 37°C.

1027 BIOLOGICAL INTERACTIONS OF QUANTUM DOT NANOPARTICLES IN SKIN AND IN HUMAN EPIDERMAL KERATINOCYTES.

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Quantum dots (QD) nanoparticles have novel optical properties for biomedical applications, but little is known about their skin permeability and interaction with cells. Water-soluble QD621 coated with polyethylene glycol (PEG) were topically applied to porcine skin flow-through diffusion cells to assess penetration at 1μM, 2μM and 10μM for 24h. QD were also studied in human epidermal keratinocytes (HEK) to determine cellular uptake, cytotoxicity and inflammatory potential. Laser scanning confocal microscopy depicted QD penetration restricted to the uppermost stratum corneum (SC) layers of the epidermis. Transmission electron microscopy (TEM) depicted QD localization in the SC intercellular lipid bilayers. Fluorescence and inductively coupled plasma-optical emission spectroscopy (ICP-OES) analysis of cadmium (Cd) did not detect Cd in the perfusate at any time point or concentration. HEK viability decreased significantly ($p<0.05$) with QD exposure to 1.25nM to 10nM after 24h and 48h. There was a significant increase in IL-6 at concentrations from 1.25nM to 10nM at 24h and 48h, while IL-8 increased from 2.5nM to 10nM after 24h and 48h. TEM of HEK treated with 10nM of QD621 at 24h depicted QD in cytoplasmic vacuoles and at the periphery of the cell membranes. These results indicate that skin penetration of QD621 is limited primarily to the SC layers. However, if the skin were damaged then QD could become internalized in HEK and stimulate an inflammatory response. (Supported by U.S. EPA-STAR Program #RD-8317150)

1028 ION-PAIRING EFFECTS ON SKIN ABSORPTION OF CHARGED NANOPARTICLES.

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Most nanoparticles in aqueous solutions are charged colloidals. The surface charges on the nanoparticles govern many of their chemical and biological activities. In this study, it was observed that fullerene nanoparticles (nC60) in water cannot penetrate into the stratum corneum, the primary barrier of skin. Different salts can destabilize the charged nanoparticles, but do not promote skin absorption of the nanoparticles. When an ion-pairing (IP) agent was added into the nC60 solution, fullerenes penetrated deep into the stratum corneum, observed using the tape stripping method. This is the first observation of the IP effect on skin absorption of charged nanomaterials. It is difficult to dissolve fullerenes in water. Once nC60 forms in water, it cannot be extracted easily back into organic solvents. Mild oxidant, $Mg(ClO_4)_2$, and salt can destabilize nC60 in aqueous solutions and help the solvent extraction. We hypothesized that nC60 cannot be extracted back into the solvent because of the surface charges on nC60 nanoparticles. If an IP agent is added to neutralize the charges without destabilizing the aggregates, the absorption process could be accelerated. The toluene absorption profiles of nC60 from 0.1% tetrafluoroacetic acid (TFA, an IP agent) and salt solutions supported our hypothesis. The absorption amounts from $Mg(ClO_4)_2$ and KCl solutions are lower than that from water. This is due to the fact that the salts destabilized the nC60 nanoparticles that formed bigger aggregates and were harder to be extracted into the toluene phase. When 0.1% TFA was used, 95% of the dosed amount was recovered. The IP effects on skin absorption and solvent extraction may follow the same mechanism. These effects on skin absorption could be significant for cosmetic formulation and transdermal drug delivery to manipulate (enhance or decrease) skin absorption of charged nanoparticles. (Supported by USEPA-STAR Grant GAD# R833328)

1029 EVALUATION OF QUANTUM DOT NANOPARTICLE PENETRATION IN HUMAN SKIN.

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Skin, the largest organ of the body, is a potential route of exposure to quantum dots (QD) and other engineered nanomaterials. QD nanoparticles are utilized in medicine for diagnosing, imaging, fluorescent tracking of cells and drug delivery due to their intense fluorescence and flexible surface chemistry. Penetration through human skin is unknown. QD565 are spherical and have a Cd/Se core and a ZnS shell. The hypothesis was designed to test if water-soluble QD565 of three sizes and three surface coatings could penetrate intact and tape stripped (30X) human skin. Previously, our lab has shown penetration of QD565 through porcine skin (Tox Sci 9:159-165,2006). QD 565 coated with polyethylene glycol (PEG), PEG amine or carboxylic acid surface coatings with a hydrodynamic diameter of 35, 15, 14nm respectively were topically applied to human skin in flow-through diffusion cells at 62.5 pmoles/cm^2 for 8 and 24h. Perfusate samples were collected hourly and analyzed for fluorescence and Cd by inductively coupled plasma-optical emission spectroscopy (ICP-OES). After 8 and 24h, the skin was frozen in liquid nitrogen and sectioned at 20μm. Confocal microscopy of all three surface coatings and sizes did not show penetration through skin. All three QD preparations remained on the surface of the stratum corneum. QD were not detected by fluorescence or Cd by ICP-OES in the perfusate. This study showed that diverse commercially available QD did not penetrate through human skin. QD were seen within hair follicle invaginations in all treatments. Porcine skin showed skin penetration but no perfusate absorption of all three QD with different surface coatings and different sizes. This study demonstrates that penetration of human skin and porcine skin may react differently when studying nanoparticle penetration. Unlike chemical nonparticle penetration, QD behave very differently probably due to their unique physicochemical properties. These results are important for risk assessment showing that there may be a species difference. (Supported by USEPA-STAR Program #RD-8317150)

1030 COMPARISON OF NINE VIABILITY METHODS IN HUMAN EPIDERMAL KERATINOCYTES TREATED WITH DIFFERENT NANOPARTICLES.

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Carbon-based nanomaterials (CNM), which include single-wall carbon nanotubes (SWCNT) and fullerences (C_{60}), have been studied in vitro to determine their toxicity to a number of different cell types. Recent studies have found that classical

dye-based assays used to determine cell viability may produce invalid results due to CNM/dye interactions or CNM adsorption of the dye or dye products. In this study, the viability of human epidermal keratinocytes (HEK) exposed in vitro to carbon black (CB), SWCNT, and C_{60} was quantitatively determined in 96-well plates with the assays 3-[4,5-Dimethyl-2-thiazol]-2,5-diphenyl-2H-tetrazolium bromide (MTT), neutral red (NR), alamar Blue (aB), Celltiter-Blue® (CTB), Cell 96® AQueous One (CAO), CytoTox One™ (lactate dehydrogenase, LDH), calcein AM (C), and Live/Dead (L/D). In addition, trypan blue (TB) staining of HEK was quantitated with the light microscope. The assay linearity (R^2 value) was determined with HEK that were plated at concentrations ranging from 0 to 25,000 cells per well in 96-well plates. The optimal cell concentration for most of the assays, the mid point within the linear portion of the curve, was 12,500 HEK per well. HEK were then treated with 0, 0.025, 0.05, 0.1, 0.2, and 0.4 mg/ml of each CNM for 24 h. TB, C, and L/D assays, which specifically depend on direct staining of living and/or dead cells, were very difficult to interpret due to the physical interference of the CNM with the stained cells. The results of the dye-based assays vary a great deal, depending on the adsorption of the dye by the CNM. The background was much higher in the absorbance-based (MTT, NR, CAO) than the fluorescent-based assays (aB, CTB, LDH). The results of the different assays with each type of CNM was highly variable, highlighting the problem of determining accurate cell viability.

1031 INDUCTION OF AP-1-MAPKS AND NF-KB SIGNAL PATHWAYS BY TUNGSTEN CARBIDE-COBALT PARTICLES.

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Hard metal consists of a powder mixture of tungsten carbide (WC,85%) and metallic cobalt (Co,5–15%). WC–Co was evaluated as a probable carcinogen in humans by IARC in 2003. It is believed that the toxicity and carcinogenesis of WC–Co is associated with particle size. Although nanomaterials are currently being widely used in modern technology, there is a serious lack of information concerning human health effects and environmental implications of manufactured nanomaterials. In this report, we investigated the effects of nano– (40–80 nm) and fine (40 μm) 85WC/15Co particles on AP–1–mitogen–activated protein kinase (MAPKs) and NF–kB signaling in mouse epidermal (JB6) cells. The results demonstrated that the induction of AP-1 activity by nano-WC-Co was 4-times higher than that stimulated by fine–WC–Co. Similar results were obtained for NF-kB induction. The induction of AP-1 and NF–kB activity in cultured cells was time and dose-dependent. The signal transduction pathways for AP–1 activation were also investigated and the results demonstrated that both nano- and fine–WC–Co stimulate MAPK family members, including extra-cellular signal-regulated protein kinase (ERKs), p38 kinase, and C–Jun N–terminal kinase (JNKs). The potency of nano–WC-Co on MAPKs stimulation was significantly higher than that for fine WC-Co. Electron spin resonance (ESR) studies indicated that nano–WC–Co generated more reactive oxygen species (ROS) than the fine particles when incubated with the cells. Study using ROS- sensitive dyes support the conclusion that ROS are generated by WC–Co–treated cells. N–Acetylcysteine, an antioxidant agent, decreased AP–1 activation and phosphorylation of ERKs, p38 kinase and JNKs, suggest the involvement of ROS in the mechanisms of WC–Co–induced AP–1 activation. These findings demonstrate that WC–Co induces NF–kB and AP–1–MAPKs activation, which may be mediated through ROS. The size of the particles plays a critical role in toxicity and carcinogenicity of the hard metal.

1032 *IN VITRO* PENETRATION OF DENDRIMER NANOPARTICLES INTO HUMAN SKIN.

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Nanoparticles are increasingly being used in cosmetic and consumer products for the purpose of enhancing the skin penetration and stability of other ingredients. Dendrimers are spherical, highly branched, stable nanoparticles with functional groups capable of binding other molecules. Dendrimers have been shown to increase the transdermal permeation and bioavailability of indomethacin through rat skin (Chauhan et al., 2003). There is concern about the potential for nanoparticles to increase skin absorption of ingredients that are currently considered safe in cosmetics. Therefore, we evaluated the skin absorption of polyamidoamine (PAMAM) Generation 4 (G4) and Generation 5 (G5) dendrimer nanoparticles synthesized with a covalently bound fluorescent dye, fluoreseinisothiocyanate (FITC). Aqueous solutions of G4 and G5 dendrimers at concentrations of 0.08, 0.2, and 0.4%, were applied for 24 hr to human cadaver skin assembled in diffusion cells. The extent of skin penetration was determined by localization of fluorescence in the skin layers using a laser scanning confocal microscope. After 24 hr, it appeared that most of the fluorescence from dendrimers was located in or on the surface layer of skin (stratum corneum), in the opening of hair follicles, or in folds of the skin surface. At the 0.2 and 0.4% dendrimer concentration levels, even though the fluorescence appeared predominately in the stratum corneum, there was some fluorescence in the upper region of the epidermis. No fluorescence appeared in the dermis. Therefore, it appears that the G4 and G5 dendrimers can penetrate the stratum corneum and diffuse into the upper viable skin layer.

1033 INTRAVENOUS ADMINISTRATION OF GADOLINIUM MODIFIED NANOPARTICLES TO SPRAGUE-DAWLEY RATS.

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Antibody targeting of paramagnetic MRI contrast agents offers the potential of increased sensitivity by localizing deposition to the tumor site. Gadolinium (Gd)-modified alumoxane (GadAl) nanoparticles are proposed precursors that will be used in targeting tumors via antibody conjugation. In this study, we tested the general safety of GadAl compositions and their precursor nanoparticles. Adult male Sprague-Dawley rats were treated with a single intravenous infusion (IV) of the base ceramic particle, the surface modified ceramic particle and the Gd(III) modified nanoparticles (final diameters 20 and 45nm) and observed for 7 days. The base particles induced mortality immediately upon dosing. Rats treated with two 20-nm organically modified particle compositions had reduced body weight gain, but surface modification clearly resulted in increased tolerability over the base particle. Four compositions of the Gd(III) infused material, GadAl, the penultimate form prior to antibody conjugation, were tested. All 0.1 mM Gd/kg GadAl formulations (20 and 45 nm) were well tolerated with rats showing some hypoactivity after dosing and ~10% body weight gain after 7 days. Treatment with any of three different 1 mM Gd/kg GadAl formulations induced mortality immediately upon dosing whereas one 20-nm formulation was well tolerated at 0.1 and 1 mM Gd dose levels. This formulation contained the highest surface concentration of gluconic acid surface modifier and has a relaxivity approaching 30 $mM^{-1}s^{-1}$. In summary, GadAl nanoparticles and their precursors were tested for general toxicity following IV administration. Nanoparticles containing a gluconic acid modifier may be suitable for use as MRI contrast agents. Work supported by NIBIB Grant 5 R21 EB0001486-02.

1034 INDUCTION OF MATRIX METALLOPROTEINASE 2 AND 9 BY HUMAN MONOCYTES IN RESPONSE TO DIFFERENT TYPES OF METAL NANOPARTICLES.

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Recently, many studies have shown that nanoparticles can translocate from the lungs to the circulatory system. As a particulate foreign body, nanoparticles could induce host response such as reactive oxygen species (ROS) generation, inflammatory cytokines and matrix metalloproteinase (MMP) release which play a major role in tissue destruction and remodeling. MMPs are important component in many biological and pathological processes of their ability to degrade extracellular matrix components. Human MMP-2 and MMP-9 have been demonstrated to be able to degrade several substrates, including type IV collagen, elstin and native type I and II collagents. Previous studies in our laboratory have shown differences in inflammation in rat lungs after intratracheal instillation of three different kind of metal nanoparticles (Nano-Ni, Nano-Co and Nano-TiO2) that have similar mass median aerodynamic diameters and surface areas. Our previous results also suggested that different ability of metal particles in free radical generation could underlie the difference in inflammation induced by these metal nanoparticles. Here, we propose that metal nanoparticles may activate monocytes/macrophages to produce ROS and MMPs. Our results show that Nano-Co has significantly more cytotoxic effects on human monocyte cell (U937) as compared to Nano-Ni and Nano-TiO2. Nano-Co and Nano-Ni resulted in increased expression of matrix metalloproteinase 2 (MMP-2), MMP-9 and their inhibitor TIMP-2 by both zymography and RT-PCR, but Nano-TiO2 does not. Nano-Co and Nano-Ni also can stimulate ROS generation in U937 cells. Finally, Nano-Co and Nano-Ni induce HIF-α accumulation in U937 cells. These findings strongly suggest that ROS induced by metal nanoparticles is influenced by their ability to activate MMP-2 and MMP-9.
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1035 ASSESSMENT OF THE TOXICITY OF SILVER NANOPARTICLES *IN VITRO*: A MITOCHONDRIAL PERSPECTIVE.

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Nanomaterials, which range in size from 1 to 100 nm, have been used to create unique devices at the nanoscale level possessing novel physical and chemical functional properties. The major toxicological concern is the fact that some of the manufactured nanomaterials are redox active, and some particles transport across cell membranes and especially into mitochondria. As so, evaluate their toxicity upon acute exposure is extremely necessary. In this work, we evaluated the toxicity of silver nanoparticles (40nm and 80nm) and their effects in rat liver mitochondria bioenergetics.
Wistar rat liver mitochondria have demonstrated severe alterations in the mitochondrial respiration and membrane potential capacities, in the presence of both sizes of silver nanoparticles. Our data demonstrated statistically significant decrease in the mitochondrial membrane potential, ADP-induced depolarization, Respiratory Control Ratio (RCR). Moreover, there is a clear decreased in repolarization and state 3 respiration, while lag phase is increased. Mitochondrial permeability transition (MPT), involved in several vital cellular signalling pathways was evaluated through mitochondrial swelling assays. The data indicate an increase in mitochondrial calcium sensitivity, with consequent mitochondrial permeability transition pore induction, in the presence of the nanoparticles (especially 80nm size).
Our results show that silver nanoparticles cause impairment of mitochondrial function, being toxicity dependent on particle size. Thus, mitochondrial toxicity may have a central role in the toxicity caused by exposure to silver nanoparticles.(This work was financed by EOARD, FA8655-07-3047)

1036 INVOLVEMENT OF ENDOCYTOSIS IN UPTAKE OF PHOSPHATIDYLSERINE-COATED SINGLE-WALLED CARBON NANOTUBES BY RAW 264.7 MACROPHAGES.

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Collapse of phospholipid membrane asymmetry and exposure of phosphatidylserine (PS) on the surface of apoptotic cells is essential for recognition and removal of apoptotic cells by phagocytes. Previously, we hypothesized and confirmed that coating of single walled carbon nanotubes (SWCNTs) with PS stimulated their recognition and uptake by macrophages, dendritic cells and microglia. However, the mechanisms of the uptake remain unknown. To study the uptake mechanisms of SWCNTs by phagocytic cells, RAW 264.7 macrophages were incubated with nanotubes coated with fluorescently labeled phospholipids– nitrobenzoxadiazole (NBD)-PS or NBD-phosphatidylcholine (NBD-PC) for different periods of time (12.5μg SWCNTs/0.5x10^5 cells). By using confocal microscopy, we found that NBD-PS-SWCNTs but not NBD-PC-SWCNTs were recognized and taken-up by macrophages. We also demonstrated that PS-coated nanotubes were localized in the lysosomes as evidenced by co-localization of fluorescence responses from NBD-PS-SWCNTs and Lyso-Tracker Red. The intravesicular localization of PS-SWCNTs in macrophages was confirmed by transmission electron microscopy. Further, the uptake of NBD-PS-SWCNTs was suppressed by a cocktail of endocytosis inhibitors (chlorpromazine, nystatin, genistein and brefeldin A). This suggests that endocytosis is involved in the uptake of SWCNTs by macrophages. We conclude that PS-coated SWCNT can be used for targeted delivery of specialized cargos - regulators, inhibitors into macrophages to control their functions and this strategy could be utilized for therapeutic regulation of immune responses. Supported by NIOSH OH008282, HL70809, AHA0535365N, Human Frontier Science Program.

1037 INHALED MULTIWALLED CARBON NANOTUBE INDUCTION OF IMMUNOMODULATORY CYTOKINES AND STRESS ASSOCIATED ENZYMES *IN VIVO*.

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Adult male mice were exposed for 14 consecutive days to 0.3 or 1 mg/m3 particle concentrations of multiwalled carbon nanotubes (MWCNT) or control air. Exposures to MWCNT resulted in alterations in Concanavalin A induced cytokine secretion from lymphocytes in the lung. MWCNT were visible within alveolar macrophages, however, granuloma and/or lung damage was not evident. A number of systemic immune function endpoints were assessed in the spleen. Here, it was found that antibody production in response to antigen stimulation was suppressed. T cell proliferative ability was also decreased in response to MWCNT inhalation exposures. Splenocytes were stained with cell specific antibodies to cell surface markers in order to determine if cell populations were affected by exposure. However, decreased T cell response could not be attributed to T cell cytotoxicity. In addition to upregulation of a number of cytokines, splenocytes were also assayed for altered gene expression by real time RT-PCR for prostaglandin associated enzymes. Prostaglandin synthase 2 (PTGS2) and prostaglandin E synthase 2 (PTGES2), enzymes involved in Prostaglandin E2 synthesis, were both induced in response to MWCNT inhalation. This work was funded by EPA grant # RD-83252701-0.

1038 ACID-FUNCTIONALIZED SINGLE-WALLED CARBON NANOTUBES ENHANCE CARDIAC ISCHEMIC/REPERFUSION INJURY.

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Engineered carbon nanotubes are being intensively developed for wide applications. Because of their unique light properties, nanotubes can impose some potentially toxic effects, particularly if they have been modified to express functionally reactive chemical groups on their surface to increase their dispersibility. The present study was designed to evaluate the cardiac effects of acid-functionalized single-walled carbon nanotubes (AF-SWNTs) on ischemic/reperfusion injury. We exposed mice to 40 μg of SWNTs, AF-SWNTs, or saline by intratracheal instillation. Twenty-four h later, mouse hearts were perfused using Langendorff system for 25 min prior to 20 min of global ischemia followed by 2 h of reperfusion. Recovery of left ventricular developed pressure (LVDP) and infarct size were measured at 1 and 2 h of reperfusion respectively. Coronary artery flow rate was measured before, during, and after ischemia. Hearts from AF-SWNTs-exposed mice had significantly lower functional recovery (17.8±5.5% vs. 44.0±5.6% for saline, $p<0.05$) and greater infarct size (56.0±3.3 vs. 37.5±6.8% for saline, $p<0.05$) relative to controls. Hearts from SWNTs exposed mice had no significant effects on functional recovery (28.6±3.2%) and infarct size (31.2±5.0%) relative to controls. We also found that AF-SWNTs-exposed hearts had significantly higher coronary artery flow rate before ischemia (6.2±0.4 ml/min) than that of saline (3.0±0.2, $p<0.05$ vs. AF-SWNT) and SWNT hearts (3.4±0.4, $p<0.05$ vs. AF-SWNT). To determine whether the acid-functionalized groups contributed to the cardiac toxicity, we exposed mice to ultrafine carbon black (UFCB) and acid-functionalized UFCB (AF-UFCB). We found that treatment with AF-UFCB resulted in significant pulmonary injury, but no cardiac toxicity. To examine whether the adverse cardiac effect was due to the inflammatory effect of nanotubes, we exposed mice to LPS but found no significant effects on cardiac function. This study indicates that exposure to AF-SWNTs enhanced cardiac ischemic/reperfusion injury in mice. This proposed abstract does not reflect EPA policy.

1039 CYTOTOXIC EFFECT OF SILICA NANOPARTICLES IN HUMAN IMMUNOLOGICAL CELLS.

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The current use of nanoparticles in the manufacturing industry is growing at an accelerated rate. The nanotechnology field is projected to become a $1 trillion market by 2015. Unfortunately, the knowledge about the toxic properties of nanoparticles is not growing at the same rate that applications are found for these materials. More research is needed to understand the toxic effect of nanoparticles in living organisms. The aim of this study was to investigate the extent of any toxicity induced in vitro on human peripheral blood leukocytes by silica nanoparticles. Human blood samples were exposed to 10nm and 100nm silica nanobeads. Electron Microscopy was used to visualize nanoparticles with the cells. Cell viability and cellular thiol levels were measured using flow cytometry. Distribution of beads and glutathione (GSH) were assessed by immunoelectron microscopy. Finally, silica nanobead effects on Candida-induced cytokine production in whole blood cell culture were evaluated by Luminex multianalyte profiling kit (RnDSystems). Dose dependent decreases in cell number (90, 81.2, 36.6 11 and 0.31%) were observed with increased doses (34, 69, 137, 272 and 544ppm) of 10nm silica nanobeads when expose for 1hr; 100 nm beads did not affect viability for 2 hr even at the highest dose of 1174 ppm. The immunoelectron microscopy results show that the 10 nm beads that gain access to the cells appear to be contained in lysosomes as well in the cytoplasm, and cell morphology is clearly altered. Although the 100 nm beads seemed

to be less toxic, they substantially depleted cytosolic and nuclear GSH. No significant changes in surface thiol levels were observed; only small changes were visualized in monocytes, granulocytes and natural killer cells. A general increase in cytokine levels were observed when compared to control. Results suggest that nanoparticles inhibited the in vitro response to Candida. Silica nanoparticles induced inflammation and oxidative stress in vitro. Further studies on the differential mechanisms of 10 and 100nm particles are needed

1040 STUDY OF HEPATOTOXICITY BIOMARKERS IN SWISS-WEBSTER MICE TREATED WITH CARBON NANOTUBES.

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Carbon nanotubes [CNT's] are an example of a carbon-based nanomaterial, which has won enormous popularity in nanotechnology due its unique properties and applications. Recently, nanomaterials have received enormous national attention in the creation of new types of analytical tools for biotechnology and the life sciences. Despite the wide application of nanomaterials, there is a serious lack of information concerning their impact on human health and the environment. Therefore, evaluation and characterization of their toxic potential is necessary. The main objective of this study was to determine the effect of oxidized multiwalled carbon nanotube (MWCNT) on the activities of specific liver enzymes including alkaline/acid (ALP/ACP) phosphatases, aminotransferases (ALT/AST) and histological evaluation of liver, which may be useful as biomarkers of hepatotoxicity. Three groups of five male mice each weighing 30 + 2 g were injected intraperitoneally once a day for five days with doses of 0.25, 0.5, 0.75 mg/kg bodyweight of oxidized MWCNT. A control group was also made of 5 mice. Following anaesthetization, blood specimens were immediately collected using heparinized syringes for biochemical analysis and liver for histological evaluation. Oxidized MWCNT exposure induced dose-dependent increase in the activities of both alkaline/acid phosphatases in the serum of Swiss-Webster mice. However, the highest dose 0.75 mg/kg showed statistically significant difference in the activity of both ALP and ACP compared to control. Similar results were seen in the ALT and AST activities, however, the highest doses of 0.5 and 0.75 mg/kg MWCNT showed statistically significant difference in ALT/GPT activity alone compared to control. The histological score of hepatic fibrosis was statistically higher in mice exposed to CNTs than the control. These results suggest that carbon nanotubes indeed can be very toxic at sufficiently high doses and those serum phosphatases, aminotransferases and hepatic fibrosis can be candidate biomarkers for carbon nanotube-induced hepatotoxicity in Swiss-Webster mice.

1041 ASSESSING *IN VITRO* RESPONSES OF MURINE SPLENOCYTES TO DANSYL-SILOXANE NANOPARTICLES THROUGH FLOW CYTOMETRIC METHODS.

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Nanomaterials possess a range of biological activity, some of which may be of utility in medicine. These effects depend upon physical chemical properties, but it is not always obvious whether nanomaterials exert their observed effects intra- or extracellularly. We have developed a method for visualizing nanoparticles (NP) within cells using flow cytometric methods and a NP with imbedded dye detectable by FACS without obscuring simultaneous detection of biological markers. This FACS-based method allowed us to evaluate whether the penetration of NPs into specific cells was associated with changes in molecular markers of cellular activation and costimulation. Dansyl-containing NP were formed in an emulsion where the hydrophobic phase, PDMS [high molecular weight poly(dimethylsiloxane)] was dissolved in the sol-gel precursor TEOS and ethanol, the hydrophilic phase. After adding NH_4OH to initiate the polymerization process, surfactant Dow 190 was added. Splenic lymphocytes were isolated using standard methods and cells were treated with NPs in supplemented media (with or without LPS) for 48 hours, harvested and stained with FACS antibodies before fixation and visualization on the BD LSR II FACS instrument. Individual cells (macrophages, T and B cells) were visualized containing NP. NP exposure did not induce excess cell death or apoptosis nor were there changes in the total number of cells of various phenotypes. However, NP treatment affected all cell types. In B cells, costimulatory molecule CD86 and activation marker TLR4 were both significantly increased; in macrophages, CD86 was increased; and in $CD3^+$ T cells, $CTLA4^+$ expression was increased. These results indicate that NPs may affect cell function in the absence of cytotoxicity or apoptosis, and demonstrate a novel method to image both localization of NPs and cell-level effects. Research supported by a grant from the Institute for Nanobiomedical Technology, Johns Hopkins University.

1042 NEUROTOXICITY ASSESSMENT OF SILVER-25 NANOPARTICLES: AN *IN VITRO* AND *IN VIVO* STUDY.

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Nanoparticles have recently received a great deal of attention due to their novel physicochemical characteristics and functions. Nanoparticles are small scale substances (<100 nm) useful in many fields, including biomedicine, electronics and energy production. This elicits concern for their toxicity due to the increased exposure during large-scale industry production. Our laboratory has previously demonstrated that manganese (Mn) nanoparticles induce the depletion of dopamine and its metabolites in a dopaminergic neuronal cell line (PC-12 cells), and this depletion was accompanied by an increase in reactive oxygen species (ROS) production. In the present study, the effects of silver-25 (Ag-25) nanoparticles on the brain were evaluated in vitro and in vivo. In vitro, ROS production was measured in mouse brain homogenate after exposure to Ag-25. ROS was quantitated by measuring the oxidation of 2,7-dichlorofluorescin diacetate (DCFH-DA) to 2,7-dichlorofluorescin (DCF). Dose-dependent increases in ROS production were significant at concentrations of 60, 80 and 100 μg/ml Ag-25, with the highest concentration producing a 3.5-fold increase in ROS. For the in vivo studies, adult male C57 mice were dosed with Ag-25 (0-2000 mg/kg, ip) and sacrificed after 0.5, 1, 2, or 4 hrs. Brains were rapidly removed and ROS production was measured. In vivo exposure to Ag-25 produced a 3-fold significant increase in brain ROS after 1 hr, whereas at 0.5, 2 and 4 hrs, ROS formation was similar to control values. These data suggest that Ag-25 nanoparticles may produce neurotoxicity in vitro and in vivo by generating free radical-induced oxidative stress. Future studies will evaluate the effects of these nanoparticles on gene expression.

1043 EXPRESSION CHANGES OF DOPAMINERGIC SYSTEM-RELATED GENES IN PC12 CELLS INDUCED BY MN, AG, OR CU NANOPARTICLES.

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Nanoparticles have received great attention for producing new applications due to their novel physicochemical characteristics and functions. The rapid infusion and broad material use has also elicited concern for toxicity due to increased exposure from large scale industry production. A previous study demonstrated that manganese (Mn) nanoparticles induced dopamine depletion in a dopaminergic neuronal cell line, PC12; and this depletion was accompanied by an increase in ROS production. This study was conducted to further investigate the potential neurotoxicity of Mn, silver (Ag), and copper (Cu) nanoparticles using PC12 cells. PC12 cells were treated with 10 μg/ml of either Mn-40 nm, Ag-15 nm, or Cu-40 nm nanoparticles for 24 hrs. Mn and Cu decreased dopamine and its metabolites in PC12 cells, whereas Ag had a moderate effect. The expression of 11 genes was examined using real-time RT-PCR. The glutathione peroxidase 1 (*Gpx1*), glutathione synthetase (*Gss*), and thioredoxin reductase 1 (*Txnrd1*) genes were selected to explore redox mechanisms; tyrosine hydroxylase (*Th*), monoamine oxidase A (*Maoa*), and catechol-O-methyltransferase (*Comt*) genes were chosen due to their involvement in dopamine metabolism; the dopamine transporter (*Dat*) and vesicular monoamine transporter-2 (*Vmat2*) genes were selected to examine the transport of dopamine; and the G protein-coupled receptor 37 (*Gpr37*), α-synuclein (*Snca*), and parkin (*Park2*) genes were included to investigate the mechanisms of pathogenesis. Cu induced an overexpression of *Txnrd1*, while the expression of *Gpx1* was down-regulated after Ag or Cu treatment. Mn produced down-regulation of *Th*, which may play a role in dopamine depletion. Mn also induced a down-regulation of the expression of *Park2*; while the expression of *Snca* was up-regulated after Mn or Cu treatment. These data will help to explore specific mechanisms of neurotoxicity induced by Mn, Ag, and Cu nanoparticles.

1044 TRANSCRIPT PROFILES OF METAL OXIDE NANOPARTICLE-TREATED COLON CANCER CELLS.

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Inflammatory bowel diseases are increasing Western nations and in urban areas, and can significantly increase the risk of developing colon cancer. One hypothesis is that particulate matter may exacerbate these diseases since it is estimated that individuals may intake 10^{12}-10^{13} particles/individual/day. Reports from the literature supports the notion that cells in an inflamed colon more readily uptake nanoparticles,

and others suggest that certain nanoparticles may impair replication, transcription, and cellular proliferation. We have observed a pronounced pro-inflammatory transcriptional response in human lung cells treated with amorphous nanoSiO2. Therefore, we have undertaken a study to investigate if distinct nanoparticles are taken up by inflamed colon cells and alter gene transcription in a similar manner. We have utilized 2 distinct human colon cancer cell lines, RKO and CaCo-2, as model systems to evaluate transcriptional responses to 6 manufactured nanoparticles (5 metal oxides and carbon black) using in cosmetics, dental products, sunscreen, food additives and dyes, and for which there could be considerable occupational exposure. We have modeled two states, an inflammatory state using TNF-α pretreatment and a basal state where BSA was used as a control. First, we evaluated the basal toxicity of these nanoparticles, and then we evaluated the transcriptional responses after 4 hour treatments using whole genome microarrays. The microarray data was evaluated using the multi-class Significance Analysis of Microarrays algorithm. These nanoparticles, as a group, do not demonstrate a non-specific transcriptional effect, but instead, we observe nanoparticle species specific effects, as well as particle size effects, especially with ZnO, the most toxic particle evaluated. For this short exposure, the inflammatory state (i.e. TNF-α pre-incubation) and the nanoparticles demonstrate few overlapping alterations in transcription. Therefore, we conclude that particulate matter demonstrate both nanoparticle species and cell type specific effects.

1045 ENHANCEMENT OF PLATELET ACTIVATION AND THROMBUS FORMATION BY SILVER NANOPARTICLES.

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With the booming nanotechnology, broad applications of various nanoparticles in industry and medical fields are bringing up the concerns for their human exposure and unanticipated health effects. Here we investigated whether silver nanoparticle (nano Ag), which has been extensively used for its antibacterial activity, could increase the activation of platelets, thrombus formation and ultimately the progression of cardiovascular diseases. In isolated human platelets, nano Ag induced platelet aggregation in a concentration-dependent manner, accompanied by increased serotonin secretion and surface adhesion molecule expression. In addition, nano Ag enhanced phosphatidylserine exposure to the platelet outer membrane, resulting in increased procoagulant activity, which is crucial in the accelerated blood coagulation. Remarkably, in the presence of sub-threshold level of physiological agonists such as thrombin and collagen, these effects of nano Ag were substantially potentiated, signifying the synergistic effects of nano Ag in the progression of cardiovascular diseases in susceptible patients. In FACS analysis, nano Ag-induced intracellular calcium increase appeared to play a role in these platelet activation processes. Consistent with these *in vitro* results, *in vivo* studies revealed that platelet aggregation and thrombus formation were significantly increased indeed after acute or chronic exposure to nano Ag in rats. In conclusion, nano Ag increased platelet aggregation, procoagulant activities and ultimately in accelerated thrombus formation. Notably, exposure to nano Ag through both the parental and inhalation routes *in vivo* resulted in increased platelet aggregation and thrombus formation, strongly supporting the relevance of these effects in real life. With this study, we believe that the important evidence was provided for potential adverse effects of nano materials on cardiovascular diseases.

1046 CYTOTOXICITY OF MICRO AND NANO METALLO-OXIDE: A COMPARATIVE STUDY.

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Nanomaterials exhibit unique properties directly related to the size, composition and shape of the nanostructures. Whereas the potential benefits of nanotechnologies have been welcomed, concerns have been expressed that properties such as high surface reactivity and ability to cross cell membranes may result in greater toxicity. The present study has addressed the key question which physical and chemical characteristics of nanoparticles are mostly responsible for toxicity, in order to establish the possible existence of a general mode-of-action for nanosized particles. To this purpose different nano- and micro sized metal oxides with similar equivalent spherical diameter, were evaluated in alveolar macrophages and glial cells. Cell death and activation of the inflammatory response were observed after exposure to both Cr2O3 nano (28.4 nm) and Cr2O3 micro (50 μm), but in a different manner. Cr2O3 28.4 nm (25-100 μg/ml) induced a significant macrophages death and release of TNF-α after 24h treatment. Cell death only, occurred in glial cells and the effect was less pronounced than in macrophages (significant at 50 μg/ml, % of cell death in glia: 32+3.8%; in macrophages: 82.7+26.8%). On the contrary, Cr2O3 50 μm induced both a significant death and TNF-α release at 50-100 μg/ml, but only in glial cells. TNF-α was not involved in Cr2O3 induced cell death, since no protection was observed in the presence of a TNF-α neutralizing antibody. On the contrary, phagocytic activity seems to be relevant to counteract Cr2O3 28.4 nm toxicity. Pre-incubation with Jasplakinolide, a cytoskeleton stabilizer, greatly worsen Cr2O3 28.4 nm induced macrophages death. Cr2O3 was then compared to TiO2. TiO2 27.6 nm did not affect any of the cultures tested, while glial death was observed after TiO2 44 μm (25-100 mg/ml) treatment.
Our results indicate that reduction to nanomolar dimension is not sufficient to determine the toxicological profile of a particle, but the "ingredient" of the particle and the characteristics of the cells are also relevant.

1047 INTERACTIONS OF NANO- AND MICRO-SIZED MESOPOROUS SILICA PARTICLES WITH PRIMARY HUMAN MONOCYTE-DERIVED MACROPHAGES.

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The rapid development of nanotechnology in recent years has created a myriad of novel engineered nanomaterials. Silica-based mesoporous materials have become a popular matrix for the incorporation and support of functional species, and offer great promises in biomedical applications, such as vehicles for drug delivery, in transfection devices, and so on. However, there is little information to date on the putative adverse effects of these nanomaterials on biological systems, including the immune system. Here, we studied the interactions of mesoporous silica particles in the nano (20 nm) and micro-size range (2 μm) with primary human monocyte-derived macrophages (HMDM), the professional phagocytic cells of the immune system. Structural and textural characterization of the particles was performed using powder X-ray diffraction and transmission and scanning electron microscopy. Both nano- and mico-sized particles were found to be taken up by HMDM, as evidenced by fluorescence microscopic imaging using FITC-labeled particles. No obvious cytotoxic effects of these particles were observed using the trypan blue exclusion test, the MTT assay, and Hoechst 33342 staining. However, when HMDM were pre-incubated with mesoporous silica particles, the subsequent engulfment of apoptotic target cells was impaired. Macrophage recognition and ingestion of apoptotic cell corpses, a process referred to as programmed cell clearance, is of considerable importance for the maintenance of tissue homeostasis, and the current findings are therefore relevant to future development of mesoporous silica materials for drug delivery and other biomedical applications.

1048 TOXICITY OF SILICON NANOPARTICLES AND MICROPARTICLES.

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Information on potential toxicity of nanoparticles is needed to determine if they are safe for clinical applications. Silicon (Si) nanoparticles are being actively explored as alternative fluorescent biotags. The objective of this study was to compare the toxicity and biological responses of nano-(3-4 nm) and micro-(0.2-2.0 μm) sized Si particles in RAW 264.7 murine macrophages. Cells were exposed for 24 and 48 hr to surface-oxidized nanoparticles (1 to 200 μg/ml) or microparticles (1 to 200 μg/ml) in the presence or absence of lipopolysaccharide (LPS; 1 ng/ml). Cytotoxicity was assessed using dye exclusion and MTT assays. Morphological appearance and localization of particles were examined by phase contrast and fluorescence microscopy. Cell supernatants were used to measure production of nitric oxide and the cytokines, interleukin-6 and tumor necrosis factor-α, to assess inflammatory responses to both particle sizes. No cytotoxicity or nitric oxide production was observed in cells exposed to concentrations of ≤ 20 μg/ml Si nanoparticles and ≤ 100 μg/ml Si microparticles. Inflammatory responses were observed with Si microparticles at concentrations above 10 μg/ml while cytokine production of Si nanoparticles remained the same for low concentrations (< 20 μg/ml), but decreased for concentrations of nanoparticles higher than 20 μg/ml. When macrophges were co-exposed to LPS and microparticles, there was increased production of cytokines. Fluorescence microscopy demonstrated apparent cellular uptake of Si nanoparticles. Electron microscopy studies will demonstrate if and where Si nanoparticles and microparticles are located in the macrophages. Thus, depending on the size of the particles, there are differences in cytokine release that may suggest different pathways of cell internalization and interactions between particles and macrophages.

1049 EFFECT OF CARBON NANOPARTICLE EXPOSURE ON RENAL BARRIER EPITHELIAL CELLS IN VITRO.

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The principal cells of the murine cortical collecting duct clone 4 ($mpkCCD_{cl4}$) are a model renal cell line expressing absorptive epithelia characteristics of the distal kidney tubule. They exhibit a high resistance phenotype and respond to hormonal stimuli that control salt and water homeostasis. This resistance (>1000 ohm cm^2) makes it an ideal barrier epithelium in which to study the effects of both acute (48 hour) and chronic (7 day) carbon nanoparticles (CNP) exposure. Epithelial monolayers were grown to confluency on permeable filter supports with nanoparticles added directly to the cell culture media on the apical side of the membrane for 48 hours. Electrophysiological techniques were used to monitor changes in transepithelial resistance (TER) in the treated cultures. MWCNT, SWCNT and fullerenes in concentrations from 4-200 $\mu g/cm^2$ decreased TER 30-90% in a dose dependent manner. To determine if CNP caused structural changes, the cultures were incubated for 48 hours with 21.1 $\mu g/cm^2$ MWCNT or SWCNT and examined by fluorescence microscopy. The cultures were fixed and stained to visualize actin filaments and nuclei. MWCNT and SWCNT induced an increased number and size of large multi-nucleated cells. Differential protein expression analysis by 2-DE revealed both up- and down-regulation of several proteins identified by LC-MS/MS, including intra- and extra-cellular membrane-associated proteins stathmin-like 2 and enolase (increased) ***and*** myotubularin related protein 9, gap junction alpha-8 protein coiled-coil domain-containing protein 44, zona pellucida glycoprotein 4, olfactory receptor 586, phosphomannomutase 2, RAN binding protein 1, and actin (all decreased). These results suggest significant dose-related CNP exposure effects and warrant additional study at potential occupational exposure levels. Supported by AFOSR Grant FA9550-06-1-0083.

1050 ACTIVATION OF AUTOPHAGY IN PORCINE KIDNEY CELLS BY QUANTUM DOTS.

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Concerns have been raised regarding the safety of heavy metal containing quantum dots (QD) for in vivo imaging applications. This study compared the mechanisms of toxicity of two QD species made of different core materials (CdSe vs. InGaP), but similar size (5.7 vs. 3.7 nm) and surface composition (both ZnS capped, lipid coated and pegylated). The porcine renal proximal tubule cell line (LLC-PK1) was chosen for these studies, as metal-dependent responses in these cells have been well characterized. Both QD were taken up into the LLC-PK1 cells following short-term treatment, as determined by fluorescent confocal microscopy. The CdSe QD was found to be 10-fold more toxic to the LLC-PK1 cells than InGaP, as determined by MTT assay (48h IC50 0.8 μg/mL for CdSe vs. 8.0 μg/mL for InGaP). Neither of the QD species induced appreciable oxidative stress, as determined by lipid peroxides and reduced glutathione content, suggesting that toxicity was not metal-dependent. Additionally, CdSe QD treatment did not induce a cadmium-associated increase in caspase 3 enzyme activity. The most remarkable finding was evidence of extensive autophagic activation in CdSe QD treated cells, as determined by Lysotracker Red dye uptake and TEM, that corresponded with the temporal pattern of toxicity. Evidence of increased lysosomal activity was also observed in cells treated with the less toxic InGaP QD, though autophagosome formation was less pronounced. Autophagy induction is considered a potential pathway of programmed cell death, and may be the mechanism of QD toxicity. Autophagy induction has been described for other nanoscale materials as well, and may represent a common cellular response. These data suggest that QD cytotoxicity is dependent upon properties of the particle as a whole, and not the metal core materials. Funded by NCI Contract N01-CO-12400.

1051 INDUCTION OF METALLOTHIONEIN IN PORCINE KIDNEY CELLS BY QUANTUM DOTS.

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Quantum dots (QD) are being investigated as novel in vivo imaging agents. The development of these nanoscale materials is dependent upon their safety, in addition to their imaging capabilities. Since QD are composed of potentially toxic metal core materials, the leaching of these core metals in biological systems is of great concern. The present study examined expression of the metal-inducible gene, metallothionein-IA (MT-IA), in porcine renal proximal tubular cells (LLC-PK1) treated with QD composed of different core materials, CdSe and InGaP. The LLC-PK1 cell line was chosen for these studies, as metal-induced expression of MT-IA has been previously characterized. LLC-PK1 cells were treated with both toxic and ten fold lower concentrations of QD. Expression of MT-IA was determined by multiplex real-time PCR, utilizing 18S rRNA as internal control. Relative quantification of MT-IA gene expression was calculated by the ΔΔCT method. Treatment of cells with CdSe QD was not associated with changes in MT-IA gene expression. By contrast, incubation of the LLC-PK1 cells with the InGaP QD resulted in a dramatic increase in MT-IA expression by 21 and 43 fold, at 8 and 24h, respectively. This induction of the MT-IA gene by the InGaP QD was not due to residual free metals from fabrication, and is consistent with instability of the QD upon cellular uptake. These data suggest that QDt toxicity is not necessarily associated with leaching of the metal core material. Funded by NCI Contract N01-CO-12400.

1052 TOXICITY OF CARBON AND METAL BASED NANOPARTICLES IN HEPG2 CELLS.

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Nanoparticles are gaining popular use in the biomedical field. Specifically, carbon nanotubes are being developed for drug delivery and silver nanoparticles for antibacterial applications. However, few studies have looked at their toxic effects in mammalian cells. In this study, the toxicity of carbon nanotubes and silver nanoparticles was evalutated in the human hepatocyte cell-line HepG2 and their effects on the cytochrome P450 enzymes (CYP). Initially, toxicity was studied using the MTT assay to evaluate mitochondrial function and the HepG2 cells were dosed with 10, 25, 50, and 100 μg/mL of single-walled carbon nanotubes (SWCNT), multi-walled carbon nanotubes (MWCNT), uncoated-silver (25 nm), and polysaccharide coated-silver (10 nm) for 24, 48, and 72 hours. SWCNT, MWCNT, and uncoated-silver (25nm) produced significant toxicity at all concentrations. However, the polysaccharide coated-silver (10 nm) nanoparticles did not produce any significant toxicity until after 72 hours. Additionally, the effects of these nanoparticles on P450 enzymes were investigated through transient transfection of reporter plasmids 3A4-luc and expression plasmid SXR (Steroid and Xenobiotic Receptor) in HepG2s. Luciferase assays were used to measure induction activity. Preliminary results suggest that the nanoparticles investigated do not induce the CYP3A4 promoter.

1053 SILVER NANOPARTICLES DISRUPT GDNF SIGNALING IN MALE GERM-LINE STEM CELLS.

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Nanosized silver has found anti-microbial uses in paint, bandages, and coatings on surfaces, yet no one has addressed the potential health risk of these materials, which must be considered since they have the ability to bind proteins with high affinity. Previously, we have shown the spermatogonial stem cell line, C18-4, is a useful model for studying reproductive nanotoxicity and that proliferation of these cells is mediated by the growth factor glial cell line derived neurotrophic factor (GDNF) using a Fyn/PI3 kinase mediated signaling pathway. Based on these studies, we wanted to evaluate the effect of silver (Ag) nanoparticle size and surface chemistry on the GDNF signaling pathway in C18-4 cells. We used hydrocarbon (HC) and polysaccharide coated (PC) Ag nanoparticles with 15, 25, and 80 nm diameters. Initial MTS assays demonstrated that the PS-Ag was less toxic than the HC-Ag, but at 10 μg/ml there was not toxicity in any particle type or size. Growth curves for C18-4 cells treated with 10 μg/ml of nanoparticles and 100 ng/ml of GDNF showed that the PS-Ag particles disrupted GDNF mediated proliferation in a size dependent manner while only the HC-Ag 15nm had an effect on proliferation. Furthermore, in vitro kinase assays with a purified Fyn protein and Fyn immunoprecipitates from the C18-4 cells showed a decrease in kinase activity when treated with both the HC-Ag and the PC-Ag. Furthermore, electron microscopy has shown nanoparticle localization at the plasma membrane and uptake into the cells, indicating that the reduction in kinase activity could be occurring through several possible mechanisms: 1) GDNF being unable to bind its receptor, 2) the receptor being unable to be activated once GDNF is bound, or 3) the Fyn kinase being unable to be activated by the receptor.

1054 SILVER NANTOXICITY IS MEDIATED BY 2-MERCAPTOETHANOL IN JURKAT T CELLS.

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Silver nanoparticles (Ag^0 NPs) are being used as antibacterial agents, spoilage retardants and diagnostic tools in a variety of products and warrant investigation into possible toxic effects. Since the effects of Ag^0 NPs on immune cell function have yet

to be characterized, we wanted to evaluate the cytotoxic and immunotoxic responses of 15nm and 80nm Ag^0NP in Jurkat T cells. These cells were cultured under different media conditions: a typical medium (TM) of RPMI with 10% FBS and pen/strep and an enhanced medium (EM) with nonessential amino acids (NAA) and 2-mercaptoethonal (2ME). Jurkat T-cells were exposed to Ag^0 NP at concentrations of 0, 1, 2.5, 5, 10, 25, 50, and 100 μg/ml to evaluate size and concentration-dependent cytotoxicity, using mitochondrial function as a marker. Mitochondrial function was evaluated by measuring the degree of mitochondrial reduction of the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan by succinic dehydrogenase. In addition, cell counts were performed and percent viability was determined using trypan blue exclusion. Immunotoxicity was evaluated by assessing IL-2 secretion using ELISA assays. Cells exposed to Ag^0 NPs in TM were shown to be significantly more affected by 15nm Ag^0 as concentrations increased when compared to cells grown in EM. Furthermore, IL-2 secretion significantly increased at concentrations >10 μg/ml in the TM cells, when compared to EM and control cells. In contrast, 80nm Ag^0 NP were not significantly toxic to the cells and did not alter IL-2 secretion. Based on this data, MTT assays were performed to evaluate which component in the EM was contributing to the increased viability. TM was supplemented with NAA or 2ME and compared to the EM, which contained both. The MTT data showed that the cells grown in TM-2ME had cell viability rates comparable to the EM rates, indicating that 2ME is a critical media component for mediating Ag^0 NP cytotoxicity.

1055 FUNCTIONALLY CHARGED NANOSIZE PARTICLES DIFFERENTIALLY ACTIVATE BV2 MICROGLIA.

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The effect of particle surface charge on the biological activation of immortalized mouse microglia (BV2) was examined. Nanosize (860-950 nm) spherical polystyrene microparticles (SPM) were coated with carboxyl (COOH-) or dimethyl amino (CH3)2-N- groups to give a net negative or positive (neutral) charge, respectively. Both SPM stimulated the release of reactive species and inflammatory cytokines, increased caspase 3/7 activity, and reduced intracellular ATP levels. SPM-COOH- produced qualitatively and/or quantitatively greater change in all instances. Confocal and transmission electron microscopy revealed that the SPM were phagocytosed as singlets but formed large intra-cellular micron size aggregates. Microarray analysis indicated that SPM-COOH- and SPM-(CH3)2-N- differentially affected metabolic, transcriptional and oxidative stress pathways. Intracellular calcium increases, measured with Fura-2 ratio imaging, indicated that each SPM stimulated calcium increases through different but unidentified receptors/pathways. Increases in intracellular calcium did not occur in response to conventional TRPV vanilloid receptor agonists (e.g., acid pH, capsaicin) nor were blocked by their antagonists (e.g., capsazepine), indicating that the above cellular changes in BV2 microglia were not mediated by TRPV receptors or pH sensitive channels. Together, these data indicate that cellular and genomic expressions of oxidative stress mediated inflammation are differentially stimulated by the electrostatic surface charge carried on inert nanosize particles. Such data link the physical properties (surface charge) of nanosize particles to their (adverse) biological effects and are relevant to understanding their mode of action. DISCLAIMER: This document has been reviewed by the NHEERL but does not necessarily reflect the views of the Agency.

1056 QUANTITATIVE DETERMINATION OF C60 FULLERENE BY LC-MS/MS AND ITS TISSUE DISTRIBUTION FOLLOWING ORAL ADMINISTRATION TO RATS.

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Rapid commercialization of fullerenes and their derivatives has increased risk of occupational and environmental human exposure to these nanomaterials via oral, dermal, and inhalational uptake. However, little is known about potential impact induced by exposure to nanomaterials (fullerenes and their derivatives) for human health, and comprehensive study on toxicological evaluation and body distribution of fullerenes and their derivatives is insufficient. For correct evaluation of toxic effects of fullerenes and their derivatives observed by in vivo and in vitro assay, it is necessary to use an analytical chemical approach together with biological approach. However, only a few studies of determination of fullerenes and their derivatives in biological samples have been conducted, and there are few reports on C60 itself in biological samples. To evaluate the toxic effects and the tissue distribution of C60 in rat, a quantitative determination using LC-MS/MS, administration procedure, and extraction from tissues of rat were examined in this study. For administration solution, C60/corn oil solution, and C60/γ-cyclodextrin complex in aqueous solutions were prepared. The oral administration of C60 solutions to Wistar rat (male, 6weeks) was performed by single and repeat administrations. After single administration, the tissues of rats were collected at days 1 and 7. The concentrations of C60 in liver, kidney, spleen, and intestinal lymph of single administrated rats were determined. C60 was detected at 1.50μg/g wet wt in intestinal lymph of only one rat treated by C60/corn oil solution. However, no C60 concentrations were detected in any other tissues. Although it is possible that absorbed C60 from gastrointestinal tract moved to the intestinal lymph, further detail studies are required to verify this hypothesis.

1057 THE EQUINE ESTROGEN METABOLITE, 4-HYDROXYEQUILENIN, ACTIVATES ESTROGEN RECEPTOR-MEDIATED GENE TRANSCRIPTION AND ENHANCES GENOTOXICITY IN ER ALPHA(+) CELLS.

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There is a clear association between prolonged exposure to estrogen and an increased risk in developing cancer in the breast and endometrium. The most widely prescribed HRT represents Premarin®, which contains the equine estrogens, equilenin and equilin. We have already shown that the metabolite of equine estrogens, 4-hydroxyequilenin (4-OHEN), can be autoxidized to a reactive o-quinone, which damages cellular macromolecules eventually leading to genotoxic effects. In this study, we have tested whether 4-OHEN directly binds to the nER, which it can activate estrogen sensitive genes in the nucleus and enhance DNA damage. ER can mediate signaling pathways via the estrogen response elements (ERE) leading to gene transcription. Our results demonstrate that 4-OHEN induces ERE-luc activity in ER(+) breast cancer cells (MCF-7) with an EC50 of 27.9 nM. In order to confirm 4-OHEN's binding affinity to the ER, we have performed a chromatin immunoprecipitation assay using ERα antibody and PCR of the pS2 gene with the ERE promoter region in ERα(+) MCF-7 cells. The results show that the 4-OHEN-ER-ERE complexes could be selectively pulled down. We have also shown that 4-OHEN stimulates cell proliferation by 3-fold at 1nM or 8-fold at 100 nM in ERα(+)MCF-7 cells in 6 days, which could be completely blocked by the antiestrogen, ICI 182,780. We have further confirmed the role of ER in 4-OHEN induced DNA damage between ER(+)and ER(-) breast cancer cells using the COMET assay. ER(+) cells had significantly higher DNA damage (30%) than ER(-) cells, which could be abolished by pretreatment with the ER antagonist 4-hydroxytamoxifen. In conclusion, our data demonstrate that 4-OHEN binds to ER and gets transported to the nucleus, where it interacts with the ERE, mediating cell proliferation and enhancing DNA damage.

1058 ESTROGEN RECEPTOR ALPHA SELECTIVELY MEDIATES OXIDATIVE DNA DAMAGE INDUCED BY THE EQUINE CATECHOL ESTROGEN 4-HYDROXYEQUILENIN IN ER ALPHA POSITIVE BREAST CANCER CELLS.

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Long term exposure to estrogens increases the risk of developing breast or endometrial cancer. Metabolism of estrogens to catechols and further oxidation to highly reactive o-quinones has been correlated with the genotoxicity of estrogens. 4-Hydroxyequilenin (4-OHEN) is a major catechol metabolite of the equine estrogens present in estrogen replacement formulations. It has been shown to cause a variety of DNA damage in vitro and in vivo including oxidation, single strand breaks, and formation of bulky adducts. Previously, we have shown that breast cancer cells that contain ERα are more sensitive to 4-OHEN induced DNA damage compared to ER negative cells. In the present study, we have compared the relative amounts of oxidative DNA damage induced by 4-OHEN with the non-estrogenic p-quinone, menadione, in both ERα positive (S30) and ER negative (MDA-MB-231) breast cancer cells using COMET assay and LC-MS-MS. Both compounds induced dose-dependent DNA single strand breaks and base oxidation. Interestingly, 4-OHEN induced more DNA damage in S30 cells compared to MDA-MB-231 cells, while no difference in DNA damage was observed between these cells with menadione treatment. These results suggested that ERα might enhance catechol estrogen 4-OHEN induced DNA damage. In addition, ERα might increase the rate of 4-OHEN induced DNA damage since significantly more damage was observed

within a few minutes in S30 cells, while little DNA damage was detected in MDA-MB-231 cells under the same conditions. In contrast, when incubated with menadione, no difference in the rate of DNA damage was observed in both cells and the amount of DNA damage was essentially the same as that observed in 4-OHEN treated ER negative cells. In conclusion, we found that ERα selectively mediates and enhances 4-OHEN induced DNA damage which may suggest that ERs could contribute to the chemical carcinogenesis of estrogens.

1059 MECHANISM OF ESTROGEN CARCINOGENESIS: DEPURINATING DNA ADDUCTS AND CELLULAR TRANSFORMATION FROM REACTIVITY OF ELECTROPHILIC OXIDATIVE METABOLITES.

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17β-Estradiol (E2) is the main endogenous estrogen, and it regulates growth, differentiation and physiology of the reproductive system. There is a clear association between excessive exposure to estrogens and the development and progression of hormone-dependent gynecological cancers. Breast cancer is the primary cause of cancer-related death in nonsmoking women. Clearly, a strategy to treat, but also to prevent breast cancer is necessary, starting with elucidation of the mechanisms of estrogen carcinogenesis.

It is our hypothesis that in an ER negative environment cell transformation is caused by reaction with DNA of electrophilic oxidative estrogen metabolites; the reactivity of each metabolite and character of resulting DNA adducts control the potential for cellular transformation. We treated the immortalized, ER negative, human epithelial cells (MCF-10A) with E2, 4-OHE2 (± Catechol O-Methyl Transferase, COMT inhibitor Ro 41-0960) and 4-FE2. 4-OHE2 is the bioactivation product from P450 hydroxylation of E2. The synthetic analog 4-FE2 is the metabolically stable alternative to E2 in which catechol formation at C4 position is impeded by fluoro derivatization. MCF-10A cells were treated with each compound (1 μM) twice weekly for four weeks. The transformation ability measured as anchorage-independent growth in soft agar displayed the following trend: 4-OHE2+Ro > 4-OHE2 > E2 > 4-FE2.

Catechols can be further oxidized to quinones which can react with adenine and guanine through Michael addition; this destabilizes the glycosidic bond leading to loss of modified bases from DNA (depurination). We developed an improved solid-phase extraction method to isolate the depurinating DNA adducts. We separated and quantified those using LC-MS-MS. The induction of transformation of MCF-10A cells in vitro and the measured depurination adducts confirm the carcinogenicity of E2.

1060 COMBINED EFFECTS OF ESTROGEN AND IONIZING RADIATION ON EPIGENETIC PROCESSES IN RAT MAMMARY GLAND.

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Currently ionizing radiation (IR) is the only genotoxic agent generally accepted as a breast carcinogen. Estrogen (E2) is another well-known breast carcinogen with both initiating and promoting properties. Women with elevated E2 levels are considered to be a high-risk group for breast cancer development and would likely be exposed to diagnostic IR procedures on a more frequent basis. Similarly, many women with E2-induced breast cancer undergo the IR treatment and are exposed to relatively high X-ray doses to the healthy breast. In vitro application of both IR and estrogen led to the malignant transformation of normal breast epithelial cells. We have recently shown that E2 as well as IR exposures applied separately result in profound epigenetic dysregulation in the rat mammary gland. Do those carcinogens act synergistically to promote deleterious epigenetic alterations in vivo? The study was aimed to address this important question.

We have analyzed the nature and roles of epigenetic changes in the E2 and IR-induced breast carcinogenesis using a rat model. Female rats were assigned to one of the following treatment groups: sham treated; E2 treated; IR treated; IR and E2 treated. Animals were sacrificed at 4 weeks, 12 weeks, 18 weeks after the irradiation. We found that IR-induced global DNA hypomethylation was observed 4, 12 and 18 weeks after IR exposure. Combined IR and E2 exposure had cumulative effects on DNA hypomethylation. We also noted a loss of trimethylation of histone H4K20 and H3K9.

Furthermore, we have found that E2, IR and combined exposure resulted in the significant alterations in the levels of microRNAs. Amongst microRNAs with altered expression were the known oncogenic microRNAs - miR-17-5p, miR-106b, miR-20a, miR23. Experimentally confirmed targets for those miRNAs are E2F1 and AIB1, RB1, TGFBR2 and Notch1. The expression changes of the aforementioned miRNAs were confirmed by qRT-PCR. The cellular repercussions of the observed changes will be discussed.

1061 ROLE OF THE ARYL HYDROCARBON RECEPTOR IN MAMMARY TUMOR PROGRESSION.

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Polycyclic aromatic hydrocarbons (PAH) likely play a role in cancer through AhR (aryl hydrocarbon receptor) activation and induction of the cytochrome P450 enzymes. These enzymes metabolize at least some AhR ligands into reactive intermediates resulting in the genetic mutations that are the hallmark of cancer. Previous studies in our laboratory using murine and rat models of mammary tumorigenesis demonstrate nuclear AhR expression in primary mammary tumors consistent with constitutive AhR activity and suggestive of ongoing epigenetic signaling by the AhR. Here, we postulate that constitutively active AhR plays a role in regulating genes involved in tumor progression through epigenetic signaling, even in the absence of exogenous ligands. To test this hypothesis, AhR activity in two human mammary tumor cell lines, Hs578T and BP-1, was down-regulated by transient transfection or stable transduction of AhR repressor (AhRR) or AhR siRNA and the ability of these cells to exhibit an invasive phenotype was evaluated. Hs578T is a human malignant mammary carcinosarcoma and BP-1 is a benzo[a]pyrene-induced malignant mammary epithelial cell line, both of which exhibit invasive phenotypes in vitro and metastasis in vivo. Transient transfection or stable transduction of AhRR in either line altered colony morphology in vitro. Furthermore, transient transfection with AhR siRNA significantly reduced tumor cell invasiveness in modified Boyden chambers. These data agree with the hypothesis that constitutively active AhR is capable of contributing to tumor invasion and that environmental AhR ligands may either enhance or alter this capability.

1062 EXPOSURE TO SERUM FROM SOY-FED RATS RESULTS IN PROTEASOME-MEDIATED DEGRADATION OF ARYL HYDROCARBON RECEPTOR IN FGC-4 CELLS.

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Consumption of soy diet has been found to reduce cancer incidence in animals and is associated with reduced cancer risk in humans. Previously, we demonstrated that female Sprague-Dawley rats fed AIN-93G diets having soy protein isolate (SPI) as the sole protein source had reduced basal aryl hydrocarbon receptor (AhR) levels which reduce the downstream signaling-mediated by this receptor. In the present study, the SPI-associated AhR reduction has been studied. The SPI-effect on AhR was not observed by feeding diets containing genistein or daidzein. To elucidate the mechanism, Rat FGC-4 cells were treated with the serum obtained from rats fed SPI-containing diets. Reduced AhR levels ($P<0.05$) were observed upon 24 h exposure to SPI-serum without any changes in the levels of chaperone proteins - HSP90 and XAP2. The AhR degradation was inhibited upon treating the cells with the proteasome inhibitor, MG132, in the presence of SPI-serum and was observed to be preceded by ubiquitination of the receptor. We hypothesized that a SPI-associated factor, responsible for the AhR degradation, destabilizes the receptor by influencing its association with the chaperone proteins. Support for this hypothesis was obtained from the observation of a reduced association of XAP2 with the immunoprecipitated AhR complex. The SPI-mediated AhR degradation was observed to be preceded by nuclear translocation of the receptor and inability to heterodimerize with ARNT. The significance of this study is based on the fact that feeding SPI-containing diets antagonizes AhR signaling mediated by a novel mechanism which differs from those established by known AhR antagonists.

1063 MOLECULAR DETERMINATION OF STRESS HORMONE-MEDIATED DRUG RESISTANCE TO PACLITAXEL IN BREAST CANCER.

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Breast cancer is one of the most frequently diagnosed cancers among women. Although chemotherapy comprises part of successful treatment, as many as 50% of patients do not benefit because their breast cancer cells become resistant to the chemotherapy. Although there is good evidence that psychological distress in cancer patients equates to poor chemotherapeutic response, it is still not recognized as a factor in risk assessment of cancer treatment. Our hypothesis is that drug resistance in breast cancer may arise in part due to stress hormone-induced alterations in cancer cells. To address this hypothesis, breast cancer cells were incubated with 10-7 M paclitaxel in the absence and presence of cortisol (10-6 M), norepinephrine (NE) and epinephrine (E) (10-7 M each) and an MTT assay was performed to measure cell cytotoxicity. These data show that at 24 and 48 hr, paclitaxel cytotxicity was reversed significantly by cortisol, NE, E. Incubation with the glucocorticoid receptor

antagonist RU486 or the beta-adrenergic receptor antagonist propranolol blocked the hormonal protective effect. The effect of paclitaxel and stress hormones on cell cycle progression was determined flow cytometry. At 48 hr of paclitaxel treatment showed that the majority of cells were found to be arrested at the G2-M phase of the cell cycle. However, incubation with stress hormones partially reversed this cell cycle arrest with only 40% of cell arrested. Incubation with RU486 or propranolol blocked the negative effect of the hormones. Finally proteomic profiling using mass spectrometry-based technologies demonstrated that stress hormones significantly alter several proteins within the cell including significant upregulation of the transcriptional activator, SMRC2. Taken together, these results demonstrate that stress hormones interfere significantly with chemotherapy and play a role in inhibiting paclitaxel from inducing cell cytotoxicity and cell cycle arrest of cancer cells

1064 EPIGENETIC DYSREGULATION IN ESTROGEN-INDUCED BREAST CARCINOGENESIS.

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Breast cancer is the most common malignancy in women, and the leading cause of death among women aged 35 to 55 years. It is recognized as both a genetic and epigenetic disease. "Genetic" is defined as a heritable change in the DNA sequence, while "epigenetic" refers to the information contained in chromatin, rather than the actual DNA sequence, that defines a heritable specific gene expression pattern.
Estrogen is a well-known breast carcinogen with both initiating and promoting properties.
The sequential accumulation of various genetic changes in the genesis of breast cancer, including the estrogen-induced breast cancer, has been extensively studied, whereas the contributions of epigenetic alterations to the etiology of breast cancer have not been elucidated.
We hypothesized that epigenetic dysregulation occurs early during the estrogen-induced mammary gland carcinogenesis and may be an important contributor to the initiation, growth, and progression of breast cancer. To test our hypothesis, we studied changes of DNA methylation and histone modification in mammary gland tissue by using the estrogen-induced model of breast carcinogenesis in ACI rat, a physiologically relevant model for the study of human breast cancer. Also, we examined the relationships between DNA methylation, histone modifications and expression of microRNAs. We established that the early predisposition and development of the estrogen-induced mammary gland tumors was associated with the substantial alterations in global DNA and locus-specific DNA methylation levels, loss of histone H3K9 and H4K20 trimethylation and altered microRNA expression profiles in mammary gland. The cellular repercussions of the observed changes will be discussed. The epigenetic model of breast cancer development will be presented.
The study was supported by the Alberta Breast Cancer Research Initiative.

1065 COMPARISON OF BIOMARKERS IN HUMAN BREAST CANCER CASES AND RAT MAMMARY TUMORS.

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There is a need to understand the changes in biomarkers during breast carcinogenesis which could help in cancer chemoprevention. This could be possible by comparing the biomarkers in the well established N-Methyl Nitrosourea (MNU) induced rat mammary carcinogenesis model with human breast cancer samples in the population. Two Tissue Micro Arrays (TMA) have been made with specimens of breast tissue from breast cancer patients and non-cancer patients. Each TMA consisted of an equal number of samples from node positive and node negative breast cancer specimens in triplicate. We investigated the expression of cyclin-D1, matrix metalloprotease-9 (MMP-9), c-Myc and ornithine decarboxylase (ODC) which are downstream of pathways activated in breast cancer like mitogen activated protein kinase (MAPK), eIF4E, PI3K/AKT, and Stat3. The immunohistochemistry analysis showed upregulation of these four biomarkers in the cancer samples compared to the normal samples in both the TMAs. We have previously found that the cancer chemopreventive compound auraptene (500 ppm in diet) significantly inhibited MNU-induced rat mammary carcinogenesis. Rapamycin, the Mammalian target of rapamycin (m-TOR) inhibitor (3.33 ppm in diet) had no significant effects on tumor incidence and multiplicity, but appeared to reduce tumor size. In the rat tumor tissues ODC and proliferating cell nuclear antigen (PCNA) were overexpressed compared to normal. PCNA expression was considerably reduced in rapamycin group while there was no difference in expression of ODC. Further analysis of these and other biomarkers is crucial to the preclinical development of auraptene.

1066 DEVELOPMENT OF BREAST CANCER IN HAMSTERS AS A MODEL TO TEST ONCOLYTIC VIROTHERAPY.

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Three models of breast cancer to be used to study oncolytic virotherapy were investigated in female Syrian golden hamsters. In contrast to most other species examined, the Syrian hamster is permissive for human Adenovirus replication. Hamsters are resistant to breast cancer, and few models have been reported. To address this challenge, we gave single doses of 2 carcinogens to 2 different groups of animals, respectively; 7,12-dimethylbenz[a]anthracene (DMBA) (200mg/kg, p.o.) which is metabolized by cytochrome P450 and N-methyl-nitrosourea (MNU) (100mg/kg, i.p. bw) a carcinogen that directly alkylates DNA. The third group of animals was treated in utero with diethylstilbestrol (DES) (10mg/ kg, p.o.) to sensitize them to hormonal cancers and then dosed with DMBA (12mg/kg, p.o.) twice a week for 8 weeks, starting at 5 – 6 weeks after birth (Rustia & Shubik, Cancer Res. 39, 4636, 1979). Body weights, palpations and PET imaging for tumors were recorded. Animals were necropsied and tissues were taken for histology and H&E staining. The median survival times (in days after carcinogen treatment) were 184, 37, and 75, and incidences of any tumor were 58%, 0% and 41% for the MNU (N=12), DMBA (N=6), and DES/DMBA (N=37) study, respectively. In the MNU study, there were 4 mammary, 6 ovarian, 1 uterine, 3 melanomas and 2 tumors of unknown origin. In the DES/DMBA study, there were 2 ovarian tumors; the rest were mostly melanomas, Squamous cell carcinomas of the skin and stomach, and ascites fluid. Both DMBA studies caused significant toxicity and multiple other complications. In conclusion the MNU study was the most successful and has not previously been reported. Cell lines from these tumors are currently growing in culture and will hopefully provide a valuable resource for testing oncolytic virotherapy.

1067 MODULATION OF XENOBIOTIC-METABOLIZING ENZYMES BY CHEMOPREVENTIVE AND CHEMOTHERAPEUTIC COMPOUNDS TO SUPPRESS MAMMARY DNA ADDUCTS AND TUMORS.

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Breast cancer is a leading cause of cancer-related death among American women. The etiology of majority of breast cancers is multifactorial, of which 95% is environmental and hormonal. Polycyclic aromatic hydrocarbons (PAHs) are commonly found in our environment and appear to be carcinogenic by genotoxic mechanisms. The detection and characterization of aromatic DNA adducts related to PAHs exposure in breast cancer patients suggest an important role of DNA adducts in breast cancer. Recently, many studies have assessed the chemopreventive and chemotherapeutic potential of naturally occurring dietary substances to inhibit mammary tumors. The major mechanisms of chemoprevention involve induction of phase II detoxification enzymes and/or inhibition of phase I enzymes that are involved in the activation of certain carcinogens.

In the present study, we evaluated the chemopreventive and chemotherapeutic potencies of sulforaphane and curcumin to suppress 7,12-dimethylbenz(a)anthracene (DMBA) induced mammary DNA adduct and tumor formation in female mice. Our results show that both sulforaphane and curcumin could modulate the Phase I/Phase II enzymatic systems. Wild type (C57BL/6J) female mice were exposed to DMBA and fed with dietary compounds, sulforaphane and curcumin. Another group of mice were fed with sulforaphane and curcumin followed by treatment with DMBA. The DMBA mediated induction of mammary and hepatic CYP1A1, 1B1 and AHR gene expressions were suppressed and phase 2 enzyme levels were elevated significantly, by these dietary chemopreventive phytochemicals. Increased chemopreventive potential was observed when mice were fed with sulforaphane and curcumin before exposure to DMBA. Treatment of mice with a combination of sulforaphane and curcumin was more effective compared to treatment with single compound.

The information gained from these studies will aid in the design of more highly developed and effective breast cancer prevention and treatment strategies, involving dietary constituents.

1068 ANTI-TUMOR EFFECT OF SUBSTITUTED QUINOLINES.

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The 5-year rate of survival for many cancer patients is still below 50% and 600,000 Americans annually die of cancer, suggesting that more effective anticancer agents and delivery systems are required to target micrometastases and residual tumor cells

that cause relapse and death. Cancer cells exhibit many defects in intracellular and intercellular communication that contribute to the loss of tissue homeostasis (excess cell proliferation, invasion, and metastasis). Intercellular communication in many organs is maintained via intercellular gap junction channels (GJIC). The goal of this study is to characterize the antitumor activity of novel substituted quinolines (PQs), which have the unique ability to enhance GJIC. Based on the computational docking experiment of connexin with a library of substituted quinolines, we found that PQ1 shows a high binding affinity with connexin. Previously we have demonstrated that PQ1 alone cause growth attenuation in multiple breast cancer cell lines in a colony growth assay (cancer cell- specific growth assay conducted in soft agar). Moreover, the effect of PQ1 on these cancer cells show a 20-50% increase in GJIC, suggesting the gain of GJIC in cancer cells plays a critical role in the anti-tumor treatment. An increased in GJIC is directly related to the anti-tumor effect in human mammary cancer cell line. Recently, we have found that 100 nM of PQ1 can cause 2-fold increase in GJIC activity using dual dye coupling assay in SW620 colon cancer cells. Interestingly, a lower concentration of 10 nM PQ1 also causes 70% growth attenuation in these cells. Immunoblotting assay reveals no changes in the expression of connexin 43 in the PQ1-untreated and -treated cells. This suggests that the effect of PQ1 on phosphorylation of connexin 43 may play a role in the increase of GJIC activity. PQ1 will be assessed using surface plasmon resonance to study the binding to specific antibody-protein targets. Taken together these results strongly indicate that substituted quinolines are anti-tumor agents for multiple cancers including breast and colon cancers.

1069 THE MAMMARY EPITHELIAL CELL SPECIFIC ROLE OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR (PPAR)γ IN DMBA-MEDIATED BREAST TUMOURIGENESIS.

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Peroxisome proliferator-activated receptor (PPAR)γ plays a role in tumourigenesis. PPARγ ligands decrease human breast cancer cell growth and induce regression of chemical-induced tumours in rodents; however, the mechanism remains unknown. Previous studies with PPARγ heterozygous mice suggest PPARγ normally suppresses dimethylbenz[a]anthrancene (DMBA)-induced breast, and other, tumour progression. Using conditional mammary epithelial cell-specific PPARγ knockout mice (PPARγ-MG KO), our aims were to evaluate whether: 1) mammary epithelial cell-specific PPARγ expression normally acts to prevent DMBA-mediated breast tumourigenesis; and 2) PPARγ activation can alter DMBA-mediated tumourigenesis in PPARγ-MG KO mice. Twelve week old PPARγ-MG KO mice and their congenic wild-type controls were randomly assigned to one of two treatment groups. Group 1 mice were treated by gavage once/week for 6 weeks with 1 mg DMBA and maintained on a normal chow diet for the entire study (25 weeks total). Group 2 mice were treated similar to Group 1 with the exception of being fed a PPARγ ligand (rosiglitazone, 3mg/kg/day) supplemented normal chow diet from week 7 to the end of the study. Preliminary data suggests that PPARγ-MG KO mice are more susceptible to DMBA-mediated tumourigenesis and have decreased survival compared to wild-type controls. In addition, dietary supplementation with rosiglitazone decreased DMBA-mediated tumourigenesis in wild-type but not PPARγ-MG KO mice. These results suggest mammary epithelial cell-specific PPARγ-dependent signaling pathways may normally act to suppress DMBA-mediated tumourigenesis. These findings also support a chemopreventive role for PPARγ ligands in breast cancer.

1070 EXPOSURE TO TCDD DURING PREGNANCY DELAYS DEVELOPMENT OF DMBA-INDUCED MAMMARY TUMORS.

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TCDD is classified as a carcinogen, and its ability to promote tumor formation is established in some model systems. However in terms of breast cancer, epidemiological and animal studies are not conclusive as to whether exposure to dioxin-like chemicals promotes tumor formation or may instead be protective. It is established that TCDD exposure suppresses normal differentiation of mammary tissue, and differentiation status can influence the susceptibility of the gland to neoplastic transformation. We have shown that mice exposed to TCDD during pregnancy have impaired differentiation of mammary tissue, including decreased branching and poor development of lobulo-alveolar structures. Since the normal differentiation of epithelial structures induced by pregnancy is considered protective against subsequent neoplastic transformation, we hypothesized that the TCDD-treated mice would be more susceptible to chemical carcinogenesis after parturition. To test this hypothesis, we treated mice with TCDD, or vehicle control, at three time points during pregnancy (10 ug/kg on days 0 and 7, and 5 ug/kg on day 14). Three weeks after parturition, DMBA was administered to induce formation of mammary tumors. Mice were palpated for tumors for 27 weeks, at which time greater than 90% of mice had either developed tumors or died from other causes. Contrary to our hypothesis, TCDD treatment was not associated with increased tumor formation, but instead delayed the formation of palpable tumors relative to DMBA-exposed mice treated with vehicle during pregnancy. Although formation of tumors was delayed in the TCDD-treated mice, once formed neither the volume, growth rate, or multiplicity of tumors differed between vehicle- and TCDD-treated groups. One proposed mechanism to explain the protective effect of TCDD against breast cancer is decreased levels of CXCR4 and CXCL12. However we found increased expression of this chemokine and its receptor in the glands of the TCDD-treated mice. Our results show a protective effect of TCDD on mammary tumor formation, however the mechanism of the protection is not yet clear.

1071 COMPARATIVE EFFECTS OF SUBCHRONIC INHALATION EXPOSURE OF RODENTS TO DIESEL AND GASOLINE ENGINE EMISSIONS, HARDWOOD SMOKE, AND SIMULATED DOWNWIND COAL EMISSIONS.

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Identically-designed studies conducted by the National Environmental Respiratory Center to disentangle causal components provided the unique opportunity to directly compare the nature and magnitude of effects among common source-based pollution mixtures. Rodents were exposed 6 hr/day, 7 days/wk to multiple dilutions of diesel and gasoline engine emissions, hardwood smoke, and simulated downwind coal combustion emissions. Organ weights, serum chemistry, hematology, bronchoalveolar lavage, and histopathology were evaluated in F344 rats exposed 1 wk or 6 mo. Clearance of Pseudomonas from lung was evaluated in C57/BL6 mice exposed 1 wk. Heart rate and electrocardiogram were evaluated in SHR rats exposed 1 wk. Responses in proximal aorta were evaluated in ApoE-/- mice exposed 7 wk. Development of respiratory allergic responses was evaluated in BALBc mice exposed during gestation and 4 wk after birth. Exacerbation of pre-existing respiratory allergic responses were evaluated in BALBc mice exposed 3 days. Exposure effects were tested for concentration-related trends and differences from control, and significant effects were compared among atmospheres. All atmospheres caused significant effects, but the responses affected and the patterns of effects among response variables differed among the atmospheres. Relative potency among atmospheres depended on both the exposure metric and the response variable selected. No atmosphere was the most toxic for all responses. Supported by the National Environmental Respiratory Center with funding from multiple government and non-government sponsors. Contents do not reflect views of any sponsor

1072 SUBCHRONIC EPISODIC EXPOSURE OF RATS TO DIESEL PLUS OZONE INDUCES MINIMAL CARDIOPULMONARY EFFECTS.

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Diesel exhaust (DE) emissions contribute to near-road air pollution and have been shown to induce a variety of cardiovascular and pulmonary abnormalities in animals and humans. Since high ozone concentrations are often associated with increased traffic-related emissions, we postulated that long-term episodic exposure of rats to DE plus ozone would induce greater effects than exposure to individual components by themselves. We exposed male Wistar Kyoto rats (12-15 wks), nose-only, to resuspended bulk DE collected from the tail pipe of 30 kW Deutz engine (2.0 mg/m3), ozone (0.5 ppm) or combination of both, 5 h/dx1 d/wkx16 wks. Control rats were exposed to filtered air. A number of pulmonary and systemic markers of inflammation and injury were analyzed 2 d after final exposure. Cardiac effects were evaluated using the Langendorff perfusion system. Mild alveolar duct hyperplasia and inflammation were noted in all rats exposed to ozone, while particle laden macrophages were detected in DE-exposed rats. Both of these changes were apparent in rats exposed to DE plus ozone but without one exacerbating the other. The only effect noted on bronchoalveolar lavage fluid markers was a small increase in neutrophils in rats exposed to DE or ozone. This effect was diminished in rats exposed to DE plus ozone. Blood hemoglobin levels were higher whereas circulating lymphocytes decreased in rats exposed to either DE or ozone but not DE

plus ozone. There were no differences in cardiac ischemia-reperfusion injury in these groups. These data suggest that subchronic episodic inhalation of diesel or ozone individually causes minimal pulmonary inflammation and mild systemic inflammation. This effect is minimized when the exposures occur together. (Abstract does not reflect US EPA policy. This research was supported in part by EPA/UNC Co-Op Agreement #CT829471.)

1073 INHIBITION OF TYROSINE PHOSPHATASE ACTIVITY INITIATES RECEPTOR SIGNALING IN AIRWAY EPITHELIAL CELLS EXPOSED TO DIESEL EXHAUST PARTICLES.

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Exposure to particulate matter is associated with increased cardiopulmonary morbidity and mortality. Diesel exhaust particles (DEP) are a major component of PM in urban areas and may contribute to PM toxicity through a mechanism involving pulmonary inflammation. Expression of inflammatory cytokines is controlled by phosphorylation-dependent signaling cascades. These signaling pathways involve the opposing activities of tyrosine kinases and protein tyrosine phosphatases (PTPases) which function to maintain signaling quiescence. PTPases contain an invariant catalytic cysteine which is highly susceptible to electrophilic attack. DEP-associated organic compounds can generate reactive oxygen species and form protein cysteine adducts. Therefore, we hypothesize that exposure to DEP impairs PTPase activity resulting in unopposed phosphorylation-dependent signaling. Here we report that exposure to 0-30 $\mu g/cm^2$ DEP for 4 hrs induces differential activation of kinases in primary cultures of human airway epithelial cells (HAEC). Interestingly, treatment with DEP of low (L-DEP) and high (H-DEP) but not intermediate (I-DEP) organic content induces phosphorylation of the receptor tyrosine kinase EGFR. Experiments showed that EGFR phosphorylation requires EGFR kinase activity but not EGFR dimerization. The same DEP capable of inducing EGFR phosphorylation also impair PTPase activity in HAEC. Moreover, EGFR-directed dephosphorylation is similarly attenuated in HAEC treated with L-DEP and H-DEP. These data show that DEP-induced activation of EGFR-dependent signaling in HAEC is the result of a loss of PTPase activities which normally function to dephosphorylate EGFR in opposition to baseline EGFR kinase activity. These data also suggest that organic content alone is not the sole determinant of the toxicity associated with DEP exposure. THIS ABSTRACT OF A PROPOSED PRESENTATION DOES NOT NECESSARILY REFLECT EPA POLICY.

1074 LUNG INJURY IS INDUCED BY INSOLUBLE AND TOTAL BUT NOT SOLUBLE PARTICULATE MATTER (PM) COLLECTED IN MEXICO CITY.

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Exposure to ambient air PM has been associated with adverse cardiopulmonary health effects; however, causative components have not been identified. The solubility of PM constituents and their bioavalability may influence their toxicity. Chemically characterized PM_{10} and $PM_{2.5}$ from the north and PM_{10} from the south of Mexico City were tested for their in vivo acute lung toxicity (at 24 and 72h). Male Wistar Kyoto rats (14 weeks) were intratracheally instilled with either saline or one of three PM samples (soluble, insoluble or total PM suspension) at 1 mg/ml (3 mg/kg). Pulmonary injury and inflammation were determined by analysis of bronchoalveolar lavage fluid (BALF) and correlated with soluble, insoluble or total PM. All insoluble fractions and total PM suspensions induced remarkable inflammation evidenced by the increases in BALF protein, albumin, lactate dehydrogenase, and γ-glutamyl transferase. These effects were accompanied by an increase in BALF total cells and neutrophils and a reduction in macrophage population. Saline-leachable fraction of any of the PM samples did not cause significant injury or inflammation. In addition, acute lung injury and inflammation were similar for all types of PM regardless of their geographical location or size. The effects observed at 24 h were still apparent at 72 h. There were no effects on circulating inflammatory cells or hematological parameters. Thus, there were no remarkable differences in the toxicity of ambient PM collected at different locations in Mexico City. Unlike metal laden residual oil fly ash particles, the ambient PM toxicity appears to be residing primarily in the insoluble fraction. (Does not reflect US EPA policy). Supported in part by EPA/UNC cooperative agreement #CR83346301).

1075 URBAN PARTICULATE MATTER ACTIVATES CALPAIN AND TRIGGERS THE UNFOLDED PROTEIN RESPONSE.

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Inhalation of particulate air pollution (PM) is associated with a variety of cardiovascular diseases, in addition to early mortality. Previous work in our laboratory demonstrated urban PM2.5 collected from Cache Valley Utah caused the upregulation of several inflammatory mediators, as well as the upregulation and cleavage of caspase-12, a protein involved in the unfolded protein response (UPR). In this study, we investigated the effect of fine (PM2.5) and coarse (PM10) particles collected from Cache Valley on various mediators of the UPR in cultured lung cells. Human lung (BEAS-2B) cells were exposed to PM2.5 or PM10 (12.5 and 25 μg/ml) for 24 h. Lipopolysaccharide (LPS; 10 ng/ml) was used as positive control. While neither PM2.5 nor PM10 caused measurable cytotoxicity as measured by the neutral red assay, both PM types caused phosphorylation of dsRNA activated protein kinase related protein (PERK), the first step of the UPR, as well as upregulation of key chaperones such as BiP, Hsp70, and Hsp90, and the release of C-reactive protein (CRP). Exposure to both fine and coarse PM resulted in Hsp70 release. Additional indicators of cellular stress in PM-exposed cells were activation of the p38 MAPK pathway indicated by Hsp27 phosphorylation and calpain activation. However, neither PM size class induced apoptosis. PM10 was often more potent than PM2.5, possibly due to the presence of bacterial components. These results indicate that in addition to inflammation and oxidative stress, the UPR may be a mechanism by which PM exert cellular stress.

1076 90-DAY INHALATION TOXICITY STUDY WITH DISK-SHAPED MAGNESIUM POTASSIUM TITANATE PARTICLES (TERRACESS PS) IN RATS.

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Fibrous particles such as asbestos and some man-made fibers have been known to produce pulmonary carcinogenic or fibrogenic effects. We have previously reported that changing a material (Terraces TF) from a fibrous to a nonfibrous disk-shaped form resulted in a marked reduction in toxicity and a lack of fibrogenicity in rats. The present study was conducted with a similar disk-shaped material, Terracess PS, to confirm a lack of toxicity and fibrogenicity in rats. Groups of 20 male and 15 female rats each were exposed to aerosols of Terracess PS dust at concentrations of 0, 2, 10, or 50 mg/m3 for 90 days. The aerodynamic diameter (MMAD) of the aerosols ranged from 4.1 to 4.8 μm. About 20% of particle mass was less than 3 μm. The lung clearance half-times for Terracess PS were estimated to be approximately 2 ½months for the 2 and 10 mg/m3 groups and approximately 4 months for the 50 mg/m3 group. The clearance rate is somewhat faster than other low solubility, low toxicity dusts like titanium dioxide. There were no test substance-related organ-weight effects observed in the tested animals in this study. Histologic evaluation showed an exposure-related uptake of aerosol particulates by the resident pulmonary alveolar macrophages within the lung. There was no evidence of increased macrophage numbers, inflammation, or fibrosis. Rats allowed to recover for approximately one and 3 months showed migration of pulmonary macrophages into aggregates and gradual clearance of test substance. The microscopic findings were consistent with exposure to a non-pathogenic, nuisance dust. As demonstrated in this study and previous studies with similar Terracess products, the disk-shaped morphology of Terracess PS particles is thought to be a critical factor in the low toxicity and the lack of fibrogenicity in rats compared to chemically similar fibrous particles. Under these experimental conditions, the NOAEL for Terracess PS was considered to be approximately 50 mg/m3.

1077 CLASSIFICATION OF AEROSOLIZED MINERAL BASE OILS UNDER THE GLOBALLY HARMONIZED SYSTEM OF CLASSIFICATION AND LABELING OF CHEMICALS (GHS).

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Mineral base oils are petroleum-derived distillate oils that are used in lubricant products. Their composition varies with crude oil and processing, but boiling points are often in the range of 300-600°C. An appropriate classification under GHS for the acute inhalation category is needed for these substances. GHS category 4 is assigned if the 4-hr LC50 is >1.0 to =<5.0 mg/L. Category 5 is given if the

LC50 is >5 mg/L and there is mortality or significant clinical signs; otherwise the substance is not classified. Data were available from 8 acute inhalation studies with aerosolized oils. In three of these studies, groups of 20 rats (10/sex) were exposed to ~0.1, 0.5, or 2.5 mg/L. In two similar studies, one group of rats was exposed to aerosol levels of 1.0 or 2.6 mg/L. Viscosity of these 5 mineral oils ranged from 15 to 460 cSt at 40°C. Five rats/sex in each group were sacrificed 18 hours after exposure; the remaining 5/sex were sacrificed after 2 wks. Endpoints in these 5 studies included body weight, clinical signs, gross necropsy, and weights of lung (wet and dry), liver, and kidney. Lungs, nose, kidneys, liver, and thoracic lymph nodes were examined microscopically. No treatment-related changes were noted in any study relative to controls. Three additional oils with viscosities ranging from ~9 to 32 cSt at 40°C were also studied. Endpoints were periodic body weights, clinical observations, and a gross necropsy on day 14. Mean aerosol concentrations ranged from 4.0 to 5.5 mg/L. All animals survived until termination and appeared normal within the 2 wk observation period, except those exposed to the 9 cSt oil. Those animals showed a lack of grooming, reddened skin, hunched posture, and reduced body weight gains. Based on the absence of mortality or significant clinical signs at maximum aerosol levels of 2.5 to 5.5 mg/L, mineral base oils with viscosities =>15 cSt at 40°C would not be classified as acute inhalation hazards under GHS.

1078 IN VIVO EVALUATION OF LUNG STRUCTURE BY MAGNETIC RESONANCE IMAGING (MRI) FOLLOWING ELASTASE INDUCED EMPHESEMA IN RATS.

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Emphysema and chronic bronchitis comprise the clinical complex known as chronic obstructive pulmonary disease (COPD). Cigarette smoke is a primary risk factor for COPD, with emphysema resulting in tissue destruction and airway expansion. MRI was used to non-invasively assess emphysema in a rodent model. To measure the extent of airway remodeling, the apparent diffusion coefficient (ADC) of inhaled hyperpolarized ^{3}He was quantified by MRI. Groups (3/time-point) of adult male rats were intratracheally administered saline or elastase (250 U/kg) and serially imaged. ^{3}He gas was generated using spin-exchange optical pumping, and animals were anesthetized and maintained on a mechanical ventilator. A Varian UnityPlus console with a 2 T wide bore magnet was used for imaging. The ^{3}He ADC was measured from three-dimensional (3D) images of control and treated animals acquired at 1-, 2- and 3-weeks post-exposure. The body-weight normalized median global ADCs in control rats, including airways, were (in units of cm^2/s/kg) 0.288, 0.254, and 0.239, respectively, and in elastase treated rats were 0.326, 0.343, and 0.244. Although global changes were quantifiable, the overall elastase lung response was heterogeneous. For example, in 3-week elastase-treated rats ADCs in random 5 x 5 x 2 mm^3 regions, which excluded major airways, typically ranged from 0.178 to 0.455 cm^2/s/kg, indicating a wide range of disease severity. Current efforts are aimed at correlating local ADC values with lung morphometry to determine whether MRI can be used as a noninvasive tool to assess the time-course of localized disease progression in individual animals. This technology will advance the evaluation of subtle changes in lung structure resulting from exposure to a broad range of xenobiotics. Most importantly, the ability to conduct repetitive measurements in the same animal offers a unique opportunity to advance the evaluation of toxicological response and mechanism of action. (Supported by NIH/NHLBI grant R01 HL073598-01 and Battelle ILA)

1079 PROGRESSIVE PULMONARY AND PLEURAL EFFECTS OF INDIUM PHOSPHIDE IN B6C3F1 MICE: PARTICULATE INDUCED PLEURAL FIBROSIS AND PROLIFERATION.

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Indium phosphide (InP) is a synthetic metal used in the microelectronics industry. Previous chronic inhalation exposure of mice to InP particles caused alveolar/bronchiolar adenomas and carcinomas, alveolar proteinosis, chronic inflammation, pleural fibrosis, hyperplasia of the pleural mesothelium and inflammation of heart arteries (NTP 2001). Although fibers are known to cause lesions in pleural tissues, pleural effects are uncommon after inhalation of a non-fibrous particulate. This study was conducted to investigate the mechanism of InP induced pleural fibrosis and hyperplasia in male B6C3F1 mice. Mice were treated with saline, 1 or 2 mg/kg InP by oropharyngeal aspiration (OA). Pleural lavage (PLF) and bronchoalveloar lavage fluid (BALF) was collected from mice 1, 3, 14 and 28 days post-aspiration. Cell number, LDH, total protein and cytokine levels were assayed from PLF and BALF. BALF cell number demonstrated a delayed increase, and total protein and LDH were increased in a time-related manner. Complex changes in BALF cytokine levels were observed. InP induced a pronounced pleural effusion on day 28. Cytokine analysis of PLF from day 28 demonstrated increased concentrations of osteopontin, C-RP, fibrinogen, MMP-9, MIP-1g TIMP-1, VCAM-1, vWF and other cytokine/growth factors in the pleural cavity of InP treated mice. Intrapleural injection of InP or indium chloride did not demonstrate an equivalent pleural effusion at day 28. Aspiration of InP caused progressive inflammation at weeks 1, 2, and 4 and subsequent multifocal regions of severe chronic inflammation with associated proliferative pneumonia, perivascular inflammation with extensive lymphoid proliferation and extensive areas of fibrotic thickening in the visceral pleura evident at week 14. InP may cause pleural fibrosis by causing a chronic pleural effusion, secondary to lung injury, which eventually results in pleural fibrosis and pleural hyperplasia.

1080 TOXICOLOGICAL CHARACTERIZATION OF INHALED CUSTOM PEPTIDES.

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In recent years, a number of new therapeutic peptides and proteins have been developed, but they must be injected in order to avoid rapid degradation by gastrointestinal enzymes. Significant development in new drug delivery systems such as nasal and lung delivery has been growing. Unfortunately, new therapeutic macromolecules are too large to pass through the epithelial and endothelial barriers without an absorption enhancer. A preliminary experiment was performed to determine the toxicity and efficacy of a Custom Peptide when administered by inhalation exposure once daily for 5 consecutive days to Sprague-Dawley rats. The proposed use of the inhaled Custom Peptide would introduce an excipient that can be used to enhance nasal and pulmonary drug delivery in tandem with a variety of inhaled pharmaceuticals. The results of this experiment demonstrated that dose levels up to 26.0 mg/kg/day did not produce any adverse test article-related findings. The Custom Peptide was well tolerated and there were no adverse clinical observations or systemic effects detected by assessment of clinical pathology parameters or following histopathological examination of all major organs. The respiratory tract (nasal cavity, nasopharynx, larynx, trachea, carina, bronchi and lungs) was examined histologically and there were no indications of local toxicity. The stability of the Custom Peptide in dose formulation solutions (2, 10, 50 mg/mL) was determined in vitro by assessing the biological activity on epithelial cell-cell adhesion. The Custom Peptide was capable of disrupting adhesion of confluent epithelial cells.
This study was supported by the Canadian Institutes of Health Research (CIHR PPP 82569)

1081 INHALATION OF STAINLESS STEEL WELDING FUME RESULTS IN DISSIMILAR INFLAMMATORY RESPONSES IN THE LUNGS OF A/J AND C57BL/6J MICE.

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Epidemiology studies suggest that inhalation of welding fume increases lung cancer risk in welders. Stainless steel (SS) fume, in particular, contains carcinogenic chromium and nickel. However, animal studies are lacking to conclusively link exposure to an increased lung cancer risk. An ongoing study in our laboratory has been to compare the inflammatory and tumorigenic responses to welding fume in lung tumor susceptible (A/J) and resistant (C57BL/6J) mice. Age and weight-matched male mice were exposed to gas metal arc-SS welding fume at 40mg/m^3 x 3hr/d for 10 days. Control mice were exposed to filtered air. At 1, 4, and 7d after the final exposure, bronchoalveolar lavage (BAL) was done postmortem. Lung cytotoxicity (LDH activity), air-blood barrier damage (albumin), inflammatory cytokines (IFN-γ, IL-6, IL-10, IL-12p70, MCP-1, TNF-α), total BAL cell counts, and differentials (≥300 cells identified) were analyzed. Inhalation of SS fume caused significant cytotoxicity and damage at all time points in both strains. Air exposure caused no effect and BAL cells were >99% alveolar macrophages. In SS fume-exposed A/J mice, total cell numbers were increased 3.5, 5.6 and 7.6 fold compared to control at 1, 4, and 7d, respectively. The inflammatory response was dominated by an increasing neutrophilic component that reached 36% by 7d. A minor influx of macrophage/monocytes and lymphocytes (<2%) was found. No cytokines were increased at any time point in the A/J. In the C57BL/6J, total cells were increased 5.2 fold versus control and this increase was maintained among all time points. The response was dominated by macrophage/monocytes and lymphocytes (7-10%) with minimal neutrophils (<5%). MCP-1 and IL-6 were increased at all time points, which confirmed the cellular response in the C57BL/6J. In conclusion, the dissimilar responses to inhaled SS fume may be due to the genetic background of these strains. Chronic inhalation studies are planned to further evaluate these strain differences.

1082 BERYLLIUM-INDUCED SENSITIZATION AND GRANULOMATOUS LUNG DISEASE IN MURINE MODELS.

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Beryllium (Be) exposure is an occupational hazard that can cause chronic beryllium disease (CBD), an irreversible, debilitating granulomatous lung disease, in as many as 3-5% of exposed workers. CBD may be viewed as a biphasic disease: first with peripheral sensitization occurring, and then, in some, progression to pulmonary granuloma formation. To look at both aspects of the disease process, we performed a mouse ear-swelling test (MEST) to correlate with the sensitization process and a 5-month oropharyngeal aspiration study to examine pulmonary granuloma development in mice. Previously, a MEST was utilized to see if different inbred mouse strains would exhibit varying hypersensitivity responses to Be. In the present study, 21 inbred mouse strains were tested for sensitization to Be and distinct strain differences were observed with the mouse ear-swelling test with the SJL/J strain being the most sensitive while the FVB/N strain was resistant. The data were analyzed using an *in silico* approach to uncover genes associated with sensitization to beryllium and Vav3, involved in the activation of T cell receptors, became a likely candidate. In the current aspiration study, 26 inbred strains were aspirated with either 20μg of beryllium metal particles or water monthly. Clear strain differences in beryllium-induced lymphogranulomatus nodules were observed. *In silico* analysis was performed on the data and candidate genes implicated in granuloma formation were uncovered and compared to the results of the MEST study. Elucidating genes responsible for the hypersensitive phenotype and granuloma formation in mice may prove useful in learning more about the mechanisms involved in the progression of CBD.

1083 USE OF A MOUSE MODEL TO EVALUATE PULMONARY INFLAMMATION CAUSED BY FLOOR DUST FROM A WATER-DAMAGED BUILDING.

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Although the causes of building-related respiratory illness are still unclear, epidemiological research has indicated that fungi and endotoxin in floor dust are associated with such health risks. In the present study, we used a mouse model to evaluate pulmonary inflammation caused by floor dusts collected from the workstations of employees in a water-damaged office building. The dusts were tested in an endotoxin-sensitive strain of mouse - C3HeB/FeJ, and pulmonary inflammation was determined. We examined correlation among markers of inflammation and levels of endotoxin and (1→3)-β-D-glucan. Each mouse was treated with dust sample (2.5 mg/kg of body weight suspended in 40 μL of saline) by pharyngeal aspiration. Control mice received an equivalent volume of saline by aspiration. At 18 hrs after aspiration, bronchoalveolar lavage was done postmortem on lungs and the following inflammatory and lung injury markers were measured: (1) neutrophil infiltration, (2) albumin and lactate dehydrogenase levels, and (3) inflammatory cytokines levels. The results demonstrated significant dust-induced inflammation in mice. Significant positive correlations were found between endotoxin levels and IL-6 ($r=0.30$, $p<0.05$), IFN-γ ($r=0.47$, $p<0.05$) and MCP-1 ($r=0.33$, $p<0.05$). However, most inflammatory markers showed no correlation with extractable-glucan levels, except for albumin which had a negative correlation. This may be due to the fact that fractions of (1→3)-β-D-glucans which induced inflammation are generally not water soluble, but the commercially available analytical method can only measure the water extractable glucan in the dust. Alternative indicators of fungi, such as culturable fungi, may exhibit a better correlation with pulmonary inflammatory potential of floor dusts. Additional studies are ongoing using this animal model with the endotoxin-insensitive strain of mouse (C3H/HeJ) to further examine such correlations.

1084 REFINEMENT OF TRADITIONAL RESTRAINED WHOLE-BODY PLETHYSMOGRAPHY (WBP): DEVELOPMENT OF A MODULAR RESTRAINED WBP CHAMBER FOR NOSE-ONLY INHALATION EXPOSURE STUDIES IN MICE.

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Restrained WBP is used to monitor breathing frequency and tidal volume in many applications including the Standard Test Method for Estimating Sensory Irritancy of Airborne Chemicals (RD50; concentration required for a 50% depression in breathing frequency; ASTM Designation: E 981-04). Traditional restrained WBP relies on the use of a sealed chamber with a tight-fitting neck collar which isolates the exposure chamber from the body where the plethysmography takes place. Compression around the neck due to the tight-fitting collar is a recognized physiologic stressor that has been shown to cause pituitary injury. This potential for injury limits the utility of the procedure for repeated dose measurements. As a refinement to traditional restrained WBP, we developed a modular restrained WBP chamber for use in mice which was constructed specifically to eliminate the tight-fitting neck seal. Measurements of breathing frequency in CD-1 mice using this system (296±52 breaths/min) were similar to those previously reported using traditional restrained WBP. To test the utility of the newly developed modular restrained WBP chamber for use in determination of RD50, we conducted a series of experiments using well-characterized materials with established RD50 values and standard test methods (ASTM Designation: E 981-04). Data collected indicate that the new modular restrained WBP chamber can be used to determine the RD50 value of gases and vapors. The modular design permits optimal sizing of the body tube for the strain and age of mouse used and adaptation of the instrumented exposure tube to any nose-only exposure chamber system. The system can be used during single & repeated exposure studies to refine estimation of inhaled dose to individual animals. This modified WBP can be used to determine the sensory irritancy of inhaled materials, providing valuable data to aid in human risk assessment, while eliminating the physiologic stress induced by traditional systems which use a neck seal.

1085 DIFFERENTIAL PHYSICOCHEMICAL PROPERTIES OF PARTICLES FROM WOOD COMBUSTION AND TRAFFIC DETERMINE PRO-INFLAMMATORY POTENTIAL.

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The importance of different sources and the contribution of different components to effects of particulate matter still deserves investigation. Wood combustion particles have been suggested to be less potent than particles from motor vehicles. The aim of this study was to determine the pro-inflammatory potential of wood combustion particles (WCP) from different combustion phases and motor vehicle particles (MVP); to study the role of TNFα and IL-1 in cytokine release by these particles; and to investigate the importance of the organic fraction. THP-1 monocytes and A549 lung epithelial cells in contact co-culture were used in these studies. Cytokine levels were measured by ELISA. Roughly similar levels of IL-8 release were induced by MVP and WCP, whereas the levels of IL-6 tended to be higher after exposure to MVP compared to WCP. Whereas IL-1 seemed to be the primary determinant for the IL-6 levels induced by MVP, IL-1 did not seem to influence the IL-6 response to WCP. Particles from the different phases of the wood combustion process elicited roughly similar responses, but the coarse fraction of the WCP induced higher levels of cytokines than the fine fraction. The organic fraction of WCP was of greater importance for the cytokine response than the organic fraction from MVP. In contrast, the washed particles from MVP induced a greater cytokine release than the organic fraction. The toxicity of the particles included in the study was generally low. A reduction in cell number seemed to be due to inhibition of cell proliferation and not cell death. This reduction in cell number was associated with the organic fraction of the particles. In conclusion, the particles from two different sources elicited differential cytokine release in a co-culture of monocytes and lung epithelial cells. The organic fraction was the most important determinant of the effects of WCP.

1086 THE PROTECTIVE EFFECT OF THE UPPER AIRWAYS AGAINST WATER SOLUBLE IRRITANT GAS EXPOSURE – A CASE STUDY OF ACUTE AMMONIA EXPOSURE.

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Background: It is a commonly held toxicological principal that highly water soluble irritant gasses (i.e. hydrogen chloride, ammonia, sulfur dioxide, etc.) are first deposited in the upper respiratory tract, resulting in a filtering effect that protects the lower airways from damage. As exposure concentrations and durations increase, water soluble gases are able to penetrate more deeply into the respiratory tract to cause effects within the lungs. Following this toxicological principal, one would hypothesize that chronic respiratory effects from irritant gases would occur only after high-level exposures that first cause significant upper respiratory tract effects. No systematic evaluation of this hypothesis could be identified in the scientific literature.
Methods: This review examined reports of over 200 cases of acute ammonia exposure identified in the scientific literature. Reported cases with and without chronic respiratory effects were evaluated based on the severity of clinical signs at initial presentation and duration of hospital stay in order to determine the severity of exposure associated with chronic pulmonary effects.

Results: Chronic respiratory effects identified in this review included obstructive and restrictive lung disease, RADS, bronchiolitis, bronchitis, and bronchiectasis. Such effects were not consistently observed among individuals with apparently similar exposures. All of the exposures with reported chronic respiratory effects were of sufficient severity to require extended hospitalization and were associated with severe upper respiratory effects.
Conclusion: A specific acute exposure level that may result in chronic respiratory effects could not be defined from the reviewed studies. However, it is clear that such exposures result in objective clinical signs of upper and lower airway injury, and require extensive hospitalization. This case study provides "real-world" support for the protective nature of the upper respiratory tract in water soluble irritant gas exposures.

1087 LUNG RESPONSE TO COARSE PM: BIOASSAY IN MICE.

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It has been hypothesized that particulate matter (PM) elicits inflammatory and toxic responses in the lung specific to its constituents, which can vary by region, time, and particle size. To identify the mechanism of toxicity in PM collected from a rural area in the San Joaquin Valley of Central California, we studied coarse particles of 2.5 μm - 10 μm diameter (PM2.5-PM10). Potential pro-inflammatory and toxic effects of PM2.5-PM10 in the lung were investigated using intratracheally instilled mice. We determined total and differential cell profiles and inflammatory chemokines in lung lavage fluid, and biomarkers of toxicity resulting from coarse PM exposure. Responses of the mice were readily observed with doses of 25-50 μg of PM. Changes in pro-inflammatory cellular profiles and chemokines showed both dose and time response; peak responses were observed 24 hours after PM instillation, with recovery as early as 48 hours. Furthermore, birefringent particle constituents were found within macrophages and neutrophils recovered from lung lavage fluid of PM treated animals. The presence of birefringent particles was increased with exposure to the insoluble fraction of PM. Our data suggest that pro-inflammatory effects observed from coarse PM collected during the summer months from California's hot and dry Central Valley are driven largely by the insoluble components of the PM mixture, and are not caused by endotoxin.

1088 OZONE EXPOSURE EXACERBATES EOSINOPHILIC AND EPITHELIAL CELL RESPONSES IN SINUS AND NASOLACRIMAL DUCT AIRWAYS IN ALLERGIC RATS.

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Exposure to ozone, the primary oxidant in photochemical smog, enhances mucous and inflammatory cell responses during allergic airways disease in humans and in laboratory animals. We developed a rodent model of allergic sinusitis to test the hypothesis that ozone would exacerbate allergen-induced inflammatory responses in sinus airways. Ovalbumin (OVA) -sensitized rats were first exposed to 0 or 1 ppm ozone (8h/day) and then immediately challenged intranasally with 0 or 0.5% OVA (in saline) for two consecutive days (Days 1 & 2; ozone coexposure), and sacrificed on Day 3. A second group of rats were also challenged on Days 1 & 2, but then exposed to ozone on Days 4 & 5 (ozone exposure post-challenge) and sacrificed on Day 6. Nasal airways were collected and processed for histological and morphometric analysis of intraepithelial mucosubstances (IM) and eosinophil cell density. In co-exposed animals, OVA challenge caused dramatic (30-fold) increase in mucosal eosinophil numbers in the nasal septum, maxillary sinus and the nasolacrimal duct that was not affected by ozone coexposure. In post-challenge studies, OVA caused eosinophilic infiltration in sinus and septum, but not the nasolacrimal duct. Ozone exposure post-challenge enhanced eosinophil recruitment into the mucosa of the sinus (1.3-fold) nasolacrimal duct (3.5-fold) and septum (2.3-fold). Furthermore exposure to ozone post OVA challenge caused increased IM in sinus airways (6-12 fold) and septum (1.2-fold) compared to OVA challenged animals exposed to filtered air. These results demonstrate unique effects of ozone to enhance pre-existing allergic inflammation and mucous responses in disparate nasal airways, as well as suggest a role for ozone in the development and progression of clinical rhinitis, sinusitis and rhinoconjuctivitis. NIH:NCCAM PO1-AT002620

1089 EFFECTS OF *IN VITRO* EXPOSURE OF HUMAN RESPIRATORY EPITHELIAL CELLS TO FORMALDEHYDE.

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Formaldehyde is the most abundant carbonyl in ambient air. Formaldehyde is ubiquitous in the environment because it is a by-product of combustion and is produced as an atmospheric oxidation product of nearly all VOCs. It is commonly used in the medical and health fields, in the manufacturing of building materials, and is present in substantial quantities in occupational and home environments. People are likely exposed to low levels of formaldehyde on a daily basis, it has therefore been suggested that formaldehyde exposure may be linked to increased hospital admissions and increased prevalence of inflammatory diseases. However, the effects of formaldehyde on specific inflammatory mediator production is largely unknown. To evaluate the potential inflammatory effects of formaldehyde, cultured human alveolar lung cells (A549) were exposed to realistic ambient and industrial concentrations of formaldehyde in a series of increasing doses of gaseous formaldehyde, using permeation tube, or large chamber, air mixtures.
Formaldehyde concentrations were measured continuously using a permeable membrane sampler, and detected with fluorometry using an automated Hantzsch derivative reaction (Dasgupta technique). The concentrations in the 3-hr exposures were approximately 55 ppb, 140 ppb, 220 ppb, 390 ppb, and 980 ppb. Inflammation was assessed by measuring IL-6 and IL-8 and cytotoxicity was determined via detection of lactate dehydrogenase (LDH) in cell culture supernatants.
At the 55 to 220 ppb formaldehyde exposure level, there were virtually no differences between exposed cells and control cells for all endpoints. Cells exposed to 390 and 980 ppb, however, showed increases in LDH, IL-6 and IL-8 over controls. The initial data analysis shows that a step-wise response from 220 to 390 to 980 ppb was observed, indicative of a dose-response. Taken together, our preliminary results indicate that formaldehyde induces cytotoxicity and inflammatory mediator production in a dose-dependent manner.

1090 NASAL UPTAKE OF DIACETYL AND BUTYRIC ACID VAPORS.

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Occupational exposure to high levels of butter flavoring vapors (BFV) has been associated with the development of bronchiolitis obliterans. BFV contain a variety of compounds, the most prevalent being diacetyl (2,3 butanedione). Inhalation exposure of rats to diacetyl or BFV results in nasal and/or lower airway necrosis and inflammation. Diacetyl is detoxified by dicarbonyl reductase, an enzyme inhibited by butyric acid. Butyric acid is also a component of BFV raising the possibility of an inhalation dosimetric interaction. The current study was aimed at examining this possibility. Towards this end, upper respiratory tract (URT) uptake of diacetyl alone and in combination with butyric acid vapor was measured in the surgically isolated URT of the male Sprague-Dawley rat at constant velocity inspiratory flow rates of 100, 200 or 400 ml/min and exposure concentrations of 100 or 300 ppm. In vitro metabolism kinetics of diacetyl in nasal mucosal homogenates were also assessed. Diacetyl was metabolized in an NADPH-dependent manner in nasal respiratory and olfactory mucosal homogenates. The total activity in olfactory (1.8 μmol/min) exceeded that in respiratory mucosa (0.3 μmol/min). Both high and low affinity pathways were observed in both tissues. Diacetyl was scrubbed with moderately high efficiency in the URT, with uptake efficiencies of 30-75% being observed depending upon the inspiratory flow rate. Although butyric acid was without effect on URT uptake of a nonmetabolized vapor, diacetyl uptake efficiencies were significantly lower in animals exposed to both 100 ppm diacetyl and 30 ppm butyric acid (36% vs 31%, p=0.02). The selective inhibition of diacetyl uptake provides evidence that diacetyl uptake was dependent on a butyric acid sensitive metabolic pathway, e.g. dicarbonyl reductase. These results suggest that the detoxication enzyme dicarbonyl reductase may be inhibited by the butyric acid vapor present in BFV leading to altered inhalation dosimetry and, perhaps, to an enhancement of diacetyl airway toxicity due to the inhibition of detoxication.
This research supported by NIH R03ES014041

1091 COMPARING THE TOXICITY OF FRESH AND AGED DIESEL EXHAUST USING SEPARATE PARTICLE AND GASEOUS EXPOSURE SYSTEMS.

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Our previous studies have shown that atmospheric aging of gaseous pollutants modifies the chemical composition of air pollution mixtures, ultimately affecting its toxicological potential. The goal of this current project is to determine whether aging of complex gaseous and particulate mixtures, such as diesel exhaust (DE), in the presence or absence of photochemistry, alters the toxicological potential of that mixture. A second goal was to examine the differences between multiple diesel emission sources, specifically those emitted from a 1980 Mercedes and a 2006 Volkswagen. Each freshly emitted DE sample was injected into a 120 m^3 outdoor smog chamber and aged utilizing natural sunlight and humidity. Two separate exposure systems, electrostatic aerosol *in vitro* exposure system (EAVES) and gas *in*

vitro exposure system (GIVES) were used simultaneously to expose human respiratory epithelial cells across an air-liquid interface to particulate and gas-phase components, respectively. We have compared the toxicity of unreacted "fresh" DE, dark-aged DE, and photochemically-aged DE. Following exposures, cells were analyzed for inflammatory cytokine production (IL-8) using RT-PCR. Both fresh and aged DE from both DE sources increased IL-8 levels as compared to the clean air control, with the Mercedes engine generating a greater response. In addition, our results suggest that aging significantly enhances the responses compared to fresh DE emissions in the particle phase and decreases in the gas phase (when particles are present). This suggests that the gaseous components are partitioning onto the particles during the aging process. Taken together, these results further support the importance of considering engine type and atmospheric aging when examining the toxicity of vehicle emissions.

1092 NASAL UPTAKE OF NAPHTHALENE IN THE F344 RAT.

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Naphthalene is a nasal toxicant and carcinogen in the rat. Information on inhalation dosimetry is needed to support development of a quantitative inhalation risk assessment for this compound. In the current study, upper respiratory tract (URT) uptake of naphthalene was measured in the male and female F344 rat at exposure concentrations of 3, 10 or 30 ppm under constant velocity inspiratory flow conditions at flow rates of 150 or 300 ml/min. To assess the potential importance of nasal CYP metabolism of naphthalene, groups of rats were pretreated with the CYP inhibitor 5-phenyl-1-pentyne (100 mg/kg, ip). In vitro metabolism of naphthalene was reduced by over 80% by the inhibitor. Nasal uptake of naphthalene in female rats was concentration dependent with uptake efficiencies (flow 150 ml/min) of 40, 34 and 28% being observed at inspired concentrations of 3, 10 and 30 ppm, respectively. A similar effect was observed in male rats (flow 150 ml/min) with uptake efficiencies of 53, 38 and 42% being observed at 3, 10 and 30 ppm, respectively. Uptake was more efficient in the male than female rat, likely do the larger size (220 g vs 150 g) of the male rats used in the study. Pretreatment with the inhibitor did not alter URT uptake of a non-metabolized vapor (acetone). In contrast, uptake of naphthalene was significantly reduced by pretreatment with the effect being greater at the lower inspired concentrations. Specifically, in pretreated female rats (150 ml/min) URT uptake averaged 25, 29 and 26%, at inspired concentrations of 3, 10 and 30 ppm, respectively. Thus, the concentration dependence of uptake was abolished by 5-phenyl-1-pentyne pretreatment. These results provide evidence that nasal CYP metabolism of naphthalene contributes to nasal scrubbing of this vapor and is also involved in the concentration dependence of uptake that is observed.
(Funded by the Naphthalene Coalition, including contributions from the Asphalt Institute, American Petroleum Institute, Electric Power Research Institute, Naphthalene Council and Utility Solid Waste Advocacy Group)

1093 AMBIENT PARTICULATE MATTER SUPPRESSES ALVEOLAR MACROPHAGE CYTOKINE RESPONSE TO LIPOPOLYSACCHARIDE.

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Epidemiologic evidence links ambient particulate matter (PM) exposure with cardiopulmonary mortality and morbidity, including the exacerbation of inflammatory disease and increased hospitalization for lung infections. Alveolar macrophages (AM) play an important defense role against infections via cytokine secretion and regulation. Diesel exhaust particles (DEP) suppress AM inflammatory cytokine response to lipopolysaccharide (LPS; from gram negative bacteria). We investigated if ambient PM induces similar suppression by exposing human AM in vitro to non-cytotoxic doses of Standard Reference Material (SRM) 2975 (DEP),SRM 1649(urban dust),and North Carolina(NC) PM for 24h. AM were then stimulated with LPS. 24hr post LPS, DEP, Urban Dust, and NC PM suppressed extracellular IL-8 vs. unexposed AM. Increased intracellular IL-8 retention (50-70%) induced, in part, the decreased IL-8 release. 6hr post LPS stimulation, DEP and NC PM significantly ($p<0.05$) suppressed total TNFα (intracellular + extracellular) production from AM vs. unexposed AM. TNFα release also was suppressed in DEP-exposed AM incubated with lipotechoic acid (from gram+ bacteria), but not altered with phorbol ester or calcium ionophore stimulation. Our data suggested that mechanisms of TNFα release are dissimilar among these stimulants. Ambient PM modulated AM response to LPS. PM exposure may increase susceptibility to lung infection. PM components responsible for cytokine suppression are being examined as well as whether different PM induce unique lipid "fingerprints" that may explain AM responses. (Support: NHEERL-DESE Coop. Train. in Env Sci. Research, EPA CT826513. This abstract may not represent official EPA policy)

1094 THE ROLE OF SPARC IN ASBESTOS-INDUCED PULMONARY FIBROSIS.

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Asbestos fibers are toxic substances that can cause serious health problems including cancer, asbestosis, and mesothelioma when they become airborne and are inhaled. Studies in mice have shown that after asbestos exposure, a complex network of cytokines, growth factors, and receptors are involved in initial inflammation and ensuing asbestosis. However, it has yet to be determined how genetic differences in these factors influence asbestos related disease development in humans. We have used gene expression studies in a mouse model to identify potential candidate genes involved in asbestos response, one such gene being Sparc. Sparc (secreted protein acidic and rich in cysteine) is a matricellular protein involved in tissue repair, extracellular matrix (ECM) regulation, cellular proliferation, and cellular adhesion. The ability of Sparc to regulate the ECM makes it a novel gene candidate for involvement in the fibrosis that occurs after asbestos exposure. The goal of this investigation was to determine the role of Sparc in fibrosis development after exposure to crocidolite asbestos or Libby amphibole, specifically targeting how lack of Sparc expression can influence ECM production. We speculated that Sparc is required for the accumulation of collagen seen in asbestos-induced pulmonary fibrosis. C57Bl/6 wild-type and Sparc knockout mice were instilled intratracheally with crocidolite, Libby amphibole, or saline for various durations. Through Real-Time RT-PCR, fluorescent staining, and Western Blotting we found that expression of Sparc is increased in both crocidolite and Libby amphibole treated mice compared to control. This increase in expression results in higher collagen deposits verified both visually through trichrome staining and via hydroxyproline levels. Lack of Sparc in these treated mice mitigated the level of collagen deposition. Overall, these results indicate that expression of Sparc is a significant step in the development of lung fibrosis through the modulation of ECM production. Supported by NCRR 017670.

1095 TOXICOLOGIC EFFECTS OF ACUTE 1, 2-DICHLOROETHANE (ETHYLENE DICHLORIDE; EDC) INHALATION IN F344 RATS.

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EDC is a high volume chemical primarily used as an intermediate in the production of vinyl chloride. It is a CNS depressant, potent eye and upper respiratory tract irritant and can induce lung, kidney, liver and adrenal gland damage. To assess the acute toxicologic effects of inhaled EDC, F344 rats were exposed to target concentrations of 0, 50, 200, 600 or 2000 ppm for 4 hours (h) or 0, 50, 100, or 150 ppm for 8 h and sacrificed 1 day (d) or 15 d later. This experimental design yielded a range of concentration (C) x time (T) products including one common value (800 ppm h) and one common EDC concentration (50 ppm). Experimental endpoints included body and organ weights, bronchoalveolar lavage (BAL), with analysis of total/differential cell counts, phagocytic activity, total protein and lactate dehydrogenase activity; gross pathology and histopathologic examination of nasal airways, larynx and trachea, lungs, adrenal glands, liver and kidneys. No mortality or altered BAL parameters were observed in any experimental group; however, rats exposed to 2000-ppm EDC had transient incoordination of gait immediately after exposure, modest but statistically significant alterations in adrenal, kidney, and liver weights and morphologic changes in the kidney and liver. In contrast to previously reported studies, the olfactory epithelium was the most sensitive target tissue with degeneration/necrosis observed at EDC concentrations ≥200 ppm for 4 h and ≥100 ppm for 8 h. Regeneration of the olfactory epithelium was observed 15 days after exposure to EDC. Both C and T influenced olfactory epithelial lesion location and severity. No signs of olfactory epithelial injury were detected in rats exposed to 50 ppm EDC for 4 or 8 h. Based on morphologic evidence of injury/degeneration of the olfactory epithelium, the most sensitive indicator of toxicity in this study, the No-Observed-Effect Level was 50 ppm EDC for up to 8 h in male and female F344 rats.
Funded by the HAP Task Force, a testing consortium of US EDC producers.

1096 BIOAEROSOL EMISSIONS FROM COMPOSTING GROUNDS INDUCE CYTOTOXICITY AND INFLAMMATORY RESPONSES IN RAW264.7 MACROPHAGES.

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Bioaerosol emissions from waste handling and composting plants have potential health impacts on workers and local residents. In this study we investigated the inflammatory and cytotoxic potential of airborne particle samples collected in a mu-

nicipal waste handling centre having a composting plant for biological waste. Samples were collected 1) next to composting windrow, 2) approximately 150 m away from the windrow in the perimeter of the dumping ground, and 3) in the nearby residential area, located 1 km away. Samples were collected for 48 h with a flow rate of 4 l/min on PVC filter (0.8 μm pore size). Mouse RAW264.7 macrophages were exposed to three doses (1, 5, 25 μg/ml) of collected particle samples, and the cell viability and production of proinflammatory cytokines IL-6 and TNF-alpha were measured after 24 hours. Responses induced by the dose of 5 μg/ml were selected for comparisons on the basis of dose-response studies. All the collected bioaerosol samples induced more cell death and cytokine release than respective control filter samples. The most profound effects were seen in IL-6 production: samples collected next to composting piles induced 14-times higher and those collected from the residential area 7.9 times higher IL-6 responses than control filter samples. When responses were calculated in relation to mean particle load per m3 of air, samples collected next to composting piles were the most potent inducers of proinflammatory cytokines whereas samples collected 150 m away from the composting piles and in residential area induced similar responses in mouse macrophages. These results indicate that bioaerosols present in composting grounds are potent inducers of inflammatory responses in mammalian cells and thus may be related to adverse health effects observed in individuals working in biowaste handling.

1097 COMPARISON OF LUNG INJURY FOR JP-8 *VERSUS* S-8 JET FUELS AT THRESHOLD CONCENTRATIONS.

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We studied lung injury parameters in C57BL/6 male mice exposed to either JP-8 or S-8 jet fuels at 53 mg/cubic meter for seven consecutive days in a nose-only exposure scenario. Our "in-line, real-time" jet fuel delivery system allowed us to utilize a very low jet fuel exposure concentration in order to determine the most sensitive areas of the lungs to injury from the fuel exposures. S-8 jet fuel exposure caused a significant decrease in total dynamic lung compliance compared to control values. However, there was no difference in dynamic lung compliance between the JP-8 and control groups. However, JP-8 jet fuel exposure caused a significant increase in inspiratory lung resistance. Electron micrographic analyses demonstrated a significant injury of the alveolar type II cells in JP-8 mice, but not S-8 mice, versus controls. In contrast, the small airways of the S-8 jet fuel-exposed mice showed injury in comparison to the control and JP-8 jet fuel-exposed animals. There are slight differences in the formula weights of JP-8 and S-8 jet fuels and this may partially account for the different lung injury scenarios at this threshold jet fuel concentration. The U.S. Armed Forces eventually will utilize S-8 (synthetic jet fuel) as a major fuel source and it vitally important to understand its toxicity potential (Supported by AFOSR Grant # FA9550-07-1-0142).

1098 EXTRAPULMONARY TISSUE DISTRIBUTION OF METALS FOLLOWING REPEATED LUNG EXPOSURES TO WELDING FUMES WITH DIFFERENT ELEMENTAL PROFILES.

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Welding fumes (WF) are a complex mixture of different metals. The pulmonary effects of WF have been well studied. Little is known about possible non-pulmonary effects (e.g., neurological, cardiovascular, renal). The goal was to study the translocation of metals from the lungs to other organ systems after treatment with chemically distinct WF. Fumes were collected from different welding processes: gas metal arc-mild steel (GMA-MS); manual metal arc-hardsurfacing (MMA-HS); flux-cored arc-hardsurfacing (FCA-HS). Metal analysis of WF indicated that GMA-MS was primarily composed of Fe (1.08 μg/gm) and Mn (0.32 μg/gm), whereas the Mn content of FCA-HS (2.0 μg/gm) and MMA-HS (1.8 μg/gm) was ~6x higher than in GMA-MS. FCA-HS (0.07 μg/gm) and MMA-HS (0.30 μg/gm) contained Cr which was present in only trace amounts in the GMA-MS WF. The FCA-HS and MMA-HS WF were found to be more water-soluble than the GMA-MS WF. Male Sprague-Dawley rats were intratracheally treated with 0.5 mg/rat of the different WF 1/wk x 7 or 11 wk. Controls were treated with saline. Four days after the last treatment, the animals were sacrificed, and multiple organs and discrete brain regions were recovered for metal analysis. Significant increases in lung and kidney Mn and Cr, blood Mn, and heart Mn were observed for the FCA-HS and MMA-HS groups compared to the GMA-MS and control groups after 11 wk. Lung Fe and Mn were significantly elevated in all three welding groups compared to controls after 11 wk. Slight elevations in Mn levels were observed in the striatum and cortex of brains from animals treated for 7 wk with each of the three WF compared to controls. These findings indicate that Cr and Mn, two potentially toxic metals, may translocate from the lungs to other organs after treatment with highly soluble WF generated from hardsurfacing processes. Welding particle source, composition, and solubility appear to be determinants of tranlocation to extrapulmonary tissues.

1099 DESIGN OF AN INHALATION EXPOSURE SYSTEM TO STUDY SPOT WELDING FUME CHARACTERISTICS AND BIOLOGICAL EFFECTS.

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Millions of workers worldwide are exposed to welding aerosols daily. Health effects in welders have included, metal fume fever, decreased lung function, and chronic bronchitis. Resistance spot welding (RSW) using adhesives is common in the automotive, aircraft, and appliance industries for fabricating with sheet metal where high speed, repetitive welding is needed and relatively thin section sizes are welded. Preliminary air sampling in an automotive plant during RSW detected numerous volatile organic compounds (VOCs) such as methyl methacrylate, toluene, benzene, that were also found in adhesives used in the process. The goals of this study were to develop an automated RSW generation and inhalation system for laboratory animals and to physically and chemically identify and characterize the aerosols formed. The system is divided into three different areas: (1) enclosed control room, (2) spot welder, and (3) animal exposure chamber and aerosol characterization equipment. RSW is performed on low carbon and galvanized steel (1/32" thick, up to 1-1/2" wide) conveyed from a pair of spools at a controlled rate using an automated welder equipped with two copper alloy electrodes. Adhesives commonly used during RSW are evenly dispensed at a controlled rate between the strips of metal to be welded. Welding aerosols are transported via a flexible trunk to the animal exposure chamber. Airborne emissions in the animal breathing zone are collected onto desorption tubes for identification and measurement of VOCs. Fume morphology, composition, and size are determined by electron microscopy, atomic emission spectroscopy, and particle impactors, respectively. With the development of this novel system, it is possible to investigate in an animal model the pulmonary effects of welding fume generated from RSW on metal coated with adhesives.

1100 A NOVEL METHOD FOR THE CHEMICAL CHARACTERIZATION OF GENERATED JET FUEL VAPOR AND AEROSOL FOR ANIMAL STUDIES.

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Human exposure to JP-8 (Jet Propellant 8) and other fuels through dermal and respiratory pathways is of concern for the U.S. Department of Defense (DoD). This work highlights the lack of standardized laboratory methods to generate and characterize inhalation exposures of animals to complex hydrocarbon mixtures such as jet fuel and proposes improvements for future studies. A systematic comparison of published inhalation studies is complicated by the use of different generation systems (atomizer, nebulizer, ultrasonic, bubbler, etc.) which leads to widely different fuel component concentrations in the vapor and aerosol phases. In many cases the chamber fuel atmosphere is not adequately characterized. Total aerosol concentrations using quartz or glass fiber filters are biased since most jet fuel components are extremely volatile. Studies by our group showed mass loss from 20 to 33% occurring within 2-4 minutes while sampling jet fuel aerosol using filters. Presented here is a novel method to improve the measurement of the hydrocarbon composition of jet fuel aerosol and vapor. Aerosol phase composition is obtained from the difference between measurements of total composition (vapor and aerosol) and vapor phase composition by itself. These are sampled and preserved using adsorbent material (CarboTrap, Supelco, Bellefonte, PA). Glass filters are used to remove the aerosol at flow rates (400 mL/min) and sampling durations (1 min) which limit the issues mentioned earlier. The tubes are thermally desorbed into a GC/MS to minimize loss of volatile compounds. Complete and accurate chemical characterization of generated exposures is critical for data interpretation especially considering that DoD is evaluating the use of a synthetic jet fuel (S-8) made using Fischer-Tropsch process which has significantly different compositions than JP-8. (This work was supported by AFOSR Grant 2006-0010A, VA RR&D grants C3575R and C4613L and the American Petroleum Institute.)

1101 SELECTIVITY OF NEUROPEPTIDE RELEASE FROM PULMONARY CAPSAICIN-SENSITIVE AFFERENTS IN RELATION TO DIESEL EXHAUST EXPOSURE.

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Substance P (SP) and/or calcitonin gene-related peptide (CGRP) immunoreactivity (IR) in lungs were examined in capsaicin-pretreated or intact rats exposed to the ambient and occupational levels of diesel exhaust, respectively. Although that they exhibit coexisting relationships between each other in C-fibers, only SP displays a

significant decrease in density of SP-IR in a dose-dependent manner, suggesting that SP-, not CGRP, -IR nerve fibers are involved in the regulation of the respiratory tract response to diesel exhaust exposure. Moreover, pretreatment with capsaicin significantly ruled out the diesel exhaust-induced change in SP, indicating that the response was attributed to capsaicin-sensitive C-fiber afferents. In summary, we conclude that the loss of afferent SP signaling due to the depletion of stored SP induced by repeated exposure to diesel exhaust may compromise the defensive function of the sensory C-fiber afferents.

1102 DETERMINATION OF CELL PROLIFERATION, APOPTOSIS AND CYTOKERATIN EXPRESSION IN THE EPITHELIUM OF THE DOG LARYNX.

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The larynx is a potential organ of interest in inhalation studies and is a standard tissue for histological examination on repeat dose toxicity studies. There is currently limited information on the microscopic anatomy of the normal dog canine larynx, therefore an investigative study was performed to map the epithelium of the larynx. Larynges from five, 18 month old, control Beagle dogs were collected at necropsy, fixed in formalin and processed to paraffin wax. Five cross sectional samples were cut through each larynx from the epiglottis to the first tracheal rings. Sections prepared from each block were stained with haematoxylin and eosin and by immunohistochemistry for a limited panel of cytokeratins (CK), proliferation marker Ki67 & PCNA and determination of apoptotic cells by TUNEL and cleaved Caspase 3. Ten pre-selected areas of epithelium from each larynx were examined microscopically.
The epithelium was uniformly squamous through the main body of the larynx becoming pseudo-stratified abruptly at the cranial trachea. The mean number of layers in the squamous epithelium was generally 2-4 but it was significantly thicker (5-6 layers) at the ventral epiglottis and ventral floor of the glottis. There was a strong correlation between the number of layers of epithelium and the number of Ki67 and PCNA positive nuclei/μm. All epithelia stained positively with anti-pan-cytokeratin antibody. In addition the squamous epithelium was positive for CK4 and negative for CK18. The pseudo-stratified epithelium was positive for CK4 and CK18 and the submucosal glands were positive for CK18 and negative for CK4. It was also noted that there was a significant increase in the number of apoptotic cells close to the ventral floor of the glottis.
The results provide a baseline against which potential induced toxicological changes in dog larynx epithelium can be assessed.

1103 CIGARETTE SMOKE CONDENSATE DOWNREGULATION OF LYSYL OXIDASE TRANSCRIPTION MEDIATED BY INACTIVATION OF NUCLEAR FACTOR I.

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Cigarette smoke (CS) contains more than 4800 different compounds resulting in lung lesions such as emphysema. Lysyl oxidase (LO) is a key enzyme for stabilization of the extracellular matrix by crosslinking collagen and elastin essential for organ morphogenesis and tissue repair. Our previous studies have showed downregulation of LO transcription by CS condensate (CSC), the particulate phase of CS. To elucidate mechanisms for CSC damage to the LO transcriptional regulation, we cloned the 5'-flanking region (3,979 nt) of the rat LO gene and showed the maximal promoter activity obtained from the fragment containing 804 base pairs upstream of ATG. Computational analysis revealed three nuclear factor I (NFI) binding consensus sequences (TAGGC) existing in this LO promoter region (-804/-1), of which two sites (-147/-133 and -594/-580) were functionally active determined by EMSA. Site-directed mutagenesis assays further illustrated the NFI binding sites as an enhancer element transactivating the LO gene. Only NFI-A and NFI-B isoforms were expressed in the lung fibroblasts. Their interaction with the cognate element was strongly modulated by CSC revealed by reporter gene expression and ChIP assays. NFI is a redox-sensitive transcription factor. CSC contains long-life oxidant such as tar-semiquinone that generates hydroxyl radicals and H2O2. Oxidation of NFI by CSC may be a critical mechanism for interfering with NFI binding to the LO gene, a basis for downregulation by CSC of LO gene transcription (Supported by NIH grant R01-ES11340).

1104 COX-2 REGULATES NF-κB ACTIVATION *VIA* THE RHO-A/JNK/AP-1 SIGNALING AXIS IN RENAL TUBULE CELLS.

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Renal tubular epithelial cells play a central role in the etiology of kidney injury through the elaboration of inflammatory mediators. However, little is known about the signaling mechanisms involved in this process. Here we used normal rat kidney epithelial (NRK52E) cells to identify a novel lipopolysaccharide (LPS)-induced signaling pathway in which cyclooxygenase-2 (Cox-2) expression is promoted by Rho-A-mediated AP-1 activity with consequent feedback inhibition of nuclear factor κB (NF-κB) activation via IKKβ. Inhibition of Rho-A signaling dramatically extended the duration of p65-DNA binding, IκBα phosphorylation, and IKKβ activity following LPS treatment. Prolongation of NF-κB activation was also observed in cells pretreated and/or co-transfected with the JNK inhibitor SP600125 or deletion mutants of MEKK1 (MEKK1-KD) or Jun (Jun-DN). Conversely, constitutive expression of Rho-A prevented NF-κB activation by LPS, and this effect was reversed by co-transfection with MEKK1-KD. In addition, we found that the Rho-A/JNK/AP-1 signaling axis plays a necessary role in Cox-2 expression by LPS and that this effect is independent of NF-κB activation. Moreover, prevention or inhibition of Cox-2 expression or activity resulted in persistent p65-DNA binding, IκBα phosphorylation and IKKβ activity, similar to that observed after prevention of Rho-A/JNK/AP-1 axis signaling. These findings show that Cox-2 links the RhoA/JNK/AP-1 signaling cascade to NF-κB activation, thereby defining a novel integrated model for regulation of the inflammatory response of kidney epithelial cells to LPS and potentially other external stimuli. Supported by NIH ES04696, ES07032 and ES07033.

1105 P38 MAP KINASE REGULATES SULFUR MUSTARD-INDUCED CYTOKINE PRODUCTION INDEPENDENT OF NF-κB AND P53 SIGNALING BUT DOES NOT REGULATE SULFUR MUSTARD-INDUCED CELL DEATH.

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Cutaneous and ocular injuries caused by sulfur mustard (SM, bis-(2-chloroethyl) sulfide) are characterized by severe inflammation. Studies have shown that inflammatory cytokines (including IL-1β, IL-6, IL-8, and TNFα) are produced in SM injury. NF-κB and p38MAPK (p38) signaling pathways are implicated in the production of inflammatory cytokines, and both of these pathways are activated in SM injury. However, a previous study by our laboratory demonstrated that a pharmacologic inhibitor of p38 alone effectively suppressed SM-induced expression of inflammatory cytokines in cultured keratinocytes, suggesting that p38 may play a more critical role than NF-κB in SM-induced inflammatory cytokine production. In this study, we utilized SB203580 (a small molecule p38 inhibitor) or inhibitory RNA (RNAi) targeted against p38α, the p50 subunit of NF-κB, or p53 to elucidate SM-induced signaling mechanisms in normal human epidermal keratinocytes (NHEK). NHEK were treated with SB203580 or RNAi and exposed to 200 μM SM. Analysis of culture supernatant from SM-exposed cells showed that inflammatory cytokine production was inhibited by p38α RNAi or SB203580, but not NF-κB p50 RNAi. These findings further support the critical role that p38 plays in SM-induced inflammatory cytokine production and suggest that NF-κB does not play a role in the SM-induced inflammatory response of NHEK. Inhibition of NF-κB by p50 RNAi did however partially inhibit SM-induced cell death suggesting a role for NF-κB in SM-induced apoptosis or necrosis. Surprisingly, inhibition of p53 by RNAi potentiated SM-induced cell death suggesting that the role of p53 in SM injury is in cell survival and not cell death. Western blot analysis of cell lysates showed that inhibition of any given target (p38, NF-κB, or p53) did not alter the activation status of the other targets, suggesting that any crosstalk between these pathways in SM injury is minimal or downstream of these molecules.

1106 PROTECTIVE EFFECT OF CAFFEIC ACID PHENETHYL ESTER ON TERT-BUTYL HYDROPEROXIDE-INDUCED OXIDATIVE HEPATOTOXICITY.

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Increased oxidative stress with high free radical generation has been described previously in animal models of the pathogenesis of various diseases, including acute liver injury. In the present work, we investigated protective effects of caffeic acid phenethyl ester (CAPE) on the tert-butyl hydroperoxide (t-BHP)-induced hepatotoxicity in cultured hepa1c1c7 cell and in liver of mouse. The treatment of the hepa1c1c7 cell with CAPE significantly reversed the t-BHP-induced oxidative injuries, as determined by cell cytotoxicity, lipid peroxidation and intracellular glutathione (GSH) content. In addition, CAPE provided protection from the t-BHP-induced production of intracellular reactive oxygen species and DNA damage. CAPE also inhibited 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals and the superoxide radical formation. The in vivo study showed that the pretreatment with CAPE prior to the administration of t-BHP significantly prevented the increase in the serum levels of hepatic enzyme markers (ALT and AST) and reduced oxidative

stress, such as GSH content and lipid peroxidation, in the liver. Consistently, histopathological examination of the livers showed that CAPE reduced the incidence of liver lesions induced by t-BHP. These results suggest that the protective effects of CAPE against t-BHP-induced hepatotoxicity may, at least in part, be due to its ability to scavenging free oxygen radicals, and protecting DNA damage from oxidative stress.

1107 METALLOTHIONEIN-III ENHANCES VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION THROUGH THE ACTIVATION OF HIF-1ALPHA.

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Metallothionein (MT) gene family consists of several members that are tightly regulated during development. MT-I and MT-II are expressed in many tissues, including the brain, whereas MT-III is expressed mainly in the central nervous system. It was first isolated and identified as a growth inhibitory factor on the basis of its inhibiting the growth and survival-promoting effect of Alzheimer's disease brain extracts on rat embryonic cortical neurons. VEGF, a hypoxia-inducible endothelial cell mitogen, has been characterized as a potent vascular permeability a critical factor in vasculogenesis and angiogenesis. In the present study, we investigated the effects of MT-III on the VEGF expression in the human endothelial-like ECV304 cell line. Treatment with MT-III under normoxic condition increased stabilization and transactivation of HIF-1alpha. MT-III enhanced transcriptional activity of HIF-1alpha in these reporter genes encoding hypoxia response element or VEGF promoter. Furthermore, MT-III induced the phophorylation of ERK and AKT in the regulation of HIF-1alpha stabilization. These results demonstrate that MT-III regulates HIF-1alpha action through a PI3K and ERK dependent pathway, resulting in increased VEGF expression.

1108 OSTEOPONTIN REGULATES MOUSE VASCULAR SMOOTH MUSCLE CELL DIFFERENTIATION *VIA* AN EXTRACELLULAR SIGNALING TRANSDUCTION PATHWAY.

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Vascular smooth muscle cells (vSMCs) lose differentiation features and gain uncontrolled proliferative activity during the early stages of atherosclerosis, a complex pathogenetic process that contributes to heart attacks, strokes, and gangrene of the extremities. Our previous studies have shown that osteopontin (OPN) mRNA and protein levels increased significantly upon induction of proliferative activity by allylamine (an atherogenic amine), and that this response was inhibited by antibodies directed against OPN. To further investigate the possible role of OPN in vSMCs differentiation, OPN was upregulated in primary aortic mouse vSMCs transfected with a constitutively active OPN expression plasmid. Intracellular OPN protein levels significantly increased after transfection, and OPN protein was also detected at higher levels in the culture medium. Several differentiation markers including alpha-actin, SM-22 alpha, tropomyosin and calponin were tested using this cellular model by Western blot analysis, and alpha-actin protein levels were found to be markedly decreased by OPN overexpression. Downregulation of alpha-actin was also observed upon extracellular treatment of mouse vSMCs with recombinant OPN or in cells expressing inducible OPN. These data suggest that OPN downregulates alpha-actin through an extracellular signaling transduction pathway. Due to the role of OPN in the early stages of atherosclerosis, targeting OPN directly may block the progression of atherosclerosis and provide a therapeutic benefit for diseases of the vascular wall. This work was supported in part by grants from the National Institute of Environmental Health Sciences and Philip Morris Extramural Research Program.

1109 GENE EXPRESSION DIFFERENCES IN P53-DEFICIENT DAY-9 MOUSE EMBRYOS.

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Neural tube defects (NTDs) are one of the most common human birth defects, with a prevalence of approximately 1 in 1000 live births in the United States. In animal studies, deletion of p53 leads to a significant increase in embryos that exhibit exencephaly, with a high prevalence in female embryos. Whereas several studies have closely investigated the morphological changes of p53-deficient embryos, there is no report that has studied the molecular-level alternations in p53-deficient embryos. Here we attempt to identify genes modified by deletion of p53 allele in day 9 mouse embryos. Day 9 mouse embryos from p53 heterozygous crosses were collected, genotyped, and embryos of similar genotype (+/+; +/-; -/-) were pooled. RNA from pooled samples was isolated and compared using Codelink mouse cDNA microarrays. In p53-deficient embryos, 310 genes show statistically significant alteration in gene expression of more than 2 fold compared to p53-wildtype embryos. As expected, expression of p53 and well known p53 target genes, such as p21 and cyclin G1, are significantly downregulated in p53-deficient embryos. In contrast, expression of other p53 target genes, such as Noxa and Puma, are unchanged, suggesting that the expression of these p53 target genes can be regulated by transcription factors other than p53. We also identified three downregulated genes (Prkaca, Ptpn11, and Csk) that are known to cause NTDs when they are deleted, indicating possible contribution of these genes to NTDs observed in p53-deficient embryos. In addition, a DNA methylation enzyme, Dnmt1, which is known to be important for X-chromosome inactivation, was also significantly downregulated, and this alteration may help to explain the female predominance of exencephalic fetuses among p53 deficient offspring. Thus, our microarray data have further defined the developmental role of p53, and have identified genes that may be related to the induction of NTDs in p53-deficient mice.

1110 TCDD DISRUPTS THE WNT SIGNALING PATHWAY IN THE DEVELOPING MOUSE KIDNEY.

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The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that mediates most of the toxic effects of 2,3,7,8-tetrachorodibenzo-p-dioxin (TCDD), including TCDD-induced hydronephrosis, a developmental abnormality of fetal kidney. While activation of the AHR is the first, obligate step of the pathway leading to TCDD-induced teratogenesis, downstream effector genes have not been identified. Because the AHR is a potent transcriptional regulator, we sought to identify genes whose expression was altered by TCDD in the developing mouse kidney. Pregnant C57BL/6 mice were injected with TCDD on gestational day 16 and sacrificed after 24 h exposure. Microarray analysis of steady-state mRNA levels in the fetal kidney revealed induction of known AHR target genes, as well as perturbation of genes important to kidney development. Cluster analysis of gene function revealed that many genes played a role in Wnt signaling, and regulation of cell cycle and growth. Pathway analysis indicated that TCDD inhibits canonical Wnt signaling in two ways: by (1) inducing negative regulators of canonical Wnt signaling and (2) inducing non-canonical Wnt signaling. *In situ* hybridization revealed localized increases in mRNA within the kidney that appear greater in magnitude than suggested by real-time PCR and microarray analysis, indicating that expression changes occur in a distinct subset of cells. Wnt pathway genes were also perturbed in fetal heart, lung, liver, skin and brain following exposure to TCDD. As the canonical Wnt signaling pathway is known to induce cell proliferation, we used BrdU to demonstrate that TCDD-induced decreases in cell proliferation occur in the same regions of the kidney where non-canonical Wnt genes are induced. Taken together, these data suggest TCDD-dependent disruption of canonical Wnt signaling. This pathway is pivotal for proper development, not only of the kidney, but also multiple other organs, and is frequently dysregulated in human cancers.

1111 TCDD ALTERS VASCULAR REMODELING IN RAT PLACENTA.

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Vascular development involves two sequential processes, vasculogenesis and angiogenesis (vascular remodeling). Vasculogenesis is mediated by vascular endothelial growth factor (VEGF) and its receptors (VEGFRs). Vascular remodeling is regulated by angiopoietin-1 and -2 and their receptor Tie2. While 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) affects vasculogenesis by altering VEGF signaling, less is known about its effects on vascular remodeling. Here, we examined effects of TCDD on vascular development in rat placenta during late gestation, a time when placental vasculogenesis has terminated but remodeling is occurring. Pregnant Holtzman rats were exposed to a single oral dose of TCDD (1,600 ng/kg) on gestation day (GD) 15 and the placenta was collected on either GD16, 18 or 20. During this period of gestation, the labyrinthine vasculature of control placenta had dilated maternal sinusoidal capillaries and fetal capillaries accompanied by reduced cellular mass and apoptotic elimination of trophoblast cells, suggesting that vascular remodeling was occurring. However, such phenomena were not observed in the placenta vasculature of TCDD-exposed pregnant rats at the same gestation period. Semi-quantitative RT-PCR showed that Tie2 mRNA, but not VEGF, VEGFRs or

angiopoietin-1 and -2 mRNAs, was reduced in TCDD-exposed placentas ($p<0.05$), suggesting that TCDD decreases remodeling of the late gestation placental vasculature. 2-D gel proteomics analysis revealed that amounts of glyceraldehyde 3-phosphate dehydrogenase, the expression of which was increased in hypoxic placenta, was significantly elevated in TCDD-exposed placenta. Furthermore, TCDD-exposed rat placenta exhibited hypoxia-related alterations in glucose kinetics including increased glycogen content and up-regulation of glucose transporter-3 (GLUT-3) mRNA level. Taken together, these findings suggest that TCDD exposure during late gestation suppresses vascular remodeling in rat placenta through the Ang/Tie2 system.

1112 EPIGALLOCATECHIN-3-GALLATE PREVENTS OBESITY-RELATED FATTY LIVER BUT IS HEPATOTOXIC AT HIGH DOSES.

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Tea polyphenols, including (-)-epigallocatechin-3-gallate (EGCG), have been studied for their preventive effects against obesity and related pathologies, and have been sold as dietary supplements for weight loss. However, case reports associate liver toxicity with consumption of green tea-based supplements. Here, we studied the preventive effects of dietary EGCG against obesity-related fatty liver disease (ORFLD), and characterized the hepatotoxic effects of high dose EGCG in mice. C57Bl6/J mice fed high fat diet (60 %kcal) for 16 weeks had increased body weight and fat, and exhibited fatty liver characterized by periportal microvesicular fat and increased hepatic triglycerides. High fat fed mice also had elevated plasma alanine aminotransferase (ALT) levels compared to low fat controls (105.2 vs 13.2 U/mL). EGCG (0.32% in the diet) decreased the incidence of ORFLD by 98% compared to high fat-fed controls. EGCG also reduced hepatic triglycerides and plasma ALT levels by 69 and 66%, respectively, compared to controls. However, we found that oral bolus administration of high dose EGCG to CF-1 mice resulted in dose-dependent hepatotoxicity. A single dose of 1500 mg/kg, i.g. EGCG increased plasma ALT levels by 138-fold and reduced survival by 85%. Daily dosing of EGCG increased hepatotoxicity. EGCG hepatotoxicity was associated with increased oxidative stress as measured by increased hepatic metallothionein and plasma 8-isoprostane. High dose EGCG also increased plasma TNFα, IL6, and monocyte chemoattractant protein 1, which have been related to hepatotoxicity. Our results indicate that moderate doses of EGCG prevent ORFLD, but higher bolus doses are hepatotoxic. Further studies on these dose and dependent effects are important given the increasing use of green tea dietary supplements, which may deliver much higher doses of EGCG than tea beverages. Supported by NIH CA125780 and CA88961.

1113 TOXIC ENDOVANILLOID ACCUMULATION IN THE LUNG LEADS TO TRPV1-MEDIATED ER STRESS AND ACUTE LUNG INJURY.

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Acute lung injury (ALI) is a consequence of systemic inflammation arising from sepsis. Currently, an effective strategy to prevent ALI in septic patients is absent and annual mortality rates remain at 25-60%. A clear mechanistic understanding of how systemic inflammation leads to ALI in septic subjects will facilitate the development of novel therapeutic agents that can ultimately be used to prevent ALI and associated mortality. It is our hypothesis that endogenous TRPV1 agonists accumulate in the lung during severe sepsis and these substances activate TRPV1 (the capsaicin receptor) and Eukaryotic Translation Initiation Factor 2α kinase-3 (EIF2Kα3)-dependent pro-oncotic endoplasmic reticulum (ER) stress pathways to cause lung cell death and ALI. In cultured human bronchial epithelial and alveolar cells, prototypical and endogenous TRPV1 agonists cause cell death via ER stress. Release of calcium from the ER through TRPV1 activates EIF2αK3 leading to the expression of Growth Arrest and DNA Damage-Inducible Transcript-3 (DDIT3, CHOP, or GADD153). GADD153 causes cell cycle arrest and oncotic cell death. Treatment of CF1 and C57BL/6J mice i.p. with lipopolysaccharide to elicit systemic inflammation, as observed in sepsis, increased lactate dehydrogenase activity in lung lavage samples and the expression of CHOP mRNA in the lung tissue. Co-treatment of mice with the TRPV1 antagonist LJO-328 or genetic deletion of TRPV1 ameliorated these responses. These results support for our hypothesis and provide new insights into key pathogenic mechanisms of ALI during severe sepsis. Discovery of TRPV1 as a mediator of ALI during sepsis will facilitate future clinical research evaluating therapeutic agents that target TRPV1 and/or the EIF2αK3 pathway. This work was supported by an NIH grant (HL069813).

1114 EFFECT OF OLEANDRIN ON BINDING AND ACTIVITY OF NA-K-ATPASE IN CRUDE CARDIAC MEMBRANE PREPARATION.

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Oleander (Nerium oleander) intoxication is one of the most common plant-associated poisonings in California, and worldwide. Ingestion of as few as 5 leaves of oleander can cause cardiac arrhythmias and death in several species, including humans. The primary cardiac glycoside in oleander is oleandrin that has been shown to inhibit Na-K-ATPase. The goal of this study was 1. To screen for drugs that could potentially prevent or reduce the binding of oleandrin to its target site, Na-K-ATPase, and 2. To screen for drugs that could limit the inhibitory effect of oleandrin on Na-K-ATPase activity. Crude cardiac membrane preparation (CCMP) was made from pig ventricles by differential centrifugation of homogenate at 6,000 g for 15 min, and then the supernatant at 100,000 g for 60 min. To demonstrate specific binding, 1nM [^{3}H]oleandrin was incubated at 37°C, for 2 hours, at a pH of 7.4, with 200 μg/ml CCMP protein. Among all 18 drugs tested, only DigiFab was effective in inhibiting oleandrin binding to the CCMP. Oleandrin also reduced the ouabain inhibitable Na-K-ATPase activity that accounted for 55-60% of the total ATPase activity. Briefly, 0.1 mg of CCMP was incubated with 1 ml solution containing 10mM ATP at 37°C, for 1 hour, pH of 7.8, and buffer that provided 5mM $MgCl_2$, 133 mM NaCl and 3mM KCl. The Na-K-ATPase activity was determined calorimetrically by measuring the amount of inorganic phosphate produced from the ATP hydrolysis. DigiFab protected the Na-K-ATPase activity of the CCMP from the inhibitory effects of oleandrin and digitoxin. This is the first study that demonstrates oleandrin binding to CCMP and confirms its inhibitory effects on the Na-K-ATPase activity. It also points out the effectiveness of DigiFab in limiting the availability of oleandrin at its target site. This study rules out direct role of other routinely used drugs in the management of oleander intoxicated patients.

1115 CHANGES IN MITOGEN-ACTIVATED PROTEIN KINASE IN CEREBELLAR GRANULE NEURONAL CULTURES BY POLYBROMINATED DIPHENYL ETHERS.

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Polybrominated diphenyl ethers (PBDEs) are used as additive flame-retardants and have been detected in human blood, adipose tissue, and breast milk; clarifying the nature of the risks posed is important for clean-up and remediation. Both in vitro and in vivo studies have shown that the effects of PBDEs are similar to the known human developmental neurotoxicants such as polychlorinated biphenyls (PCBs) on a molar basis. Previously, we reported that both PBDE mixtures as well as congeners perturbed calcium (Ca) homeostasis that is critical for the development and function of the nervous system. In the present study, we tested whether environmentally relevant PBDE mixtures and congeners affected mitogen-activated protein kinase (MAPK) pathways, which are down-stream events of calcium signaling in cerebellar granule neuronal cultures. Phosphorylated extracellular signal-regulated kinase (ERK)1/2 levels were quantitated using western blot technique with phospo-specific antibodies. Glutamate (a positive control) increased phospho-ERK in a time and concentration-dependent manner reaching maximum activation at 15-30 min of exposure and at doses >10 uM. Both Aroclor 1254 (a commercial PCB mixture) and DE-71 (a commercial PBDE mixture) elevated phospho-ERK producing maximum stimulation at 30 min and at doses >10 ug/ml; Aroclor 1254 was more efficacious than DE-71. PBDE congeners 47, 77, and 153 also increased phospo-ERK at > 3 uM suggesting that PBDE congeners are more potent than the commercial mixtures. PCB 47 increased phospho-ERK as efficaciously as PBDE 47 suggesting a common mode of action for these chemicals. These results show that PBDE mixtures and congeners activate MAPK pathway at concentrations where no significant cytotoxicity was observed, suggesting that perturbed intracellular signaling might be involved in the initiation of adverse effects related to the persistent chemicals. (This abstract does not necessarily reflect USEPA policy).

1116 EFFECT OF GINSENOSDIES RD AND RF ON ALTERING INTRACELLULAR GLUTATHIONE METABOLISM OF C6 GLIOMA CELLS.

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Ginseng is a popular herbal medicine known for its wide spectrum of pharmacological actions. It is composed of a series of saponins known as ginsenosides, which are derivatives of triterpene dammarane. Ginsenosides can be divided into protopanaxadiol (PPD: e.g. Ra, Rb, Rc, Rd, Rg3, Rh2) and protopanaxatriol (PPT:

e.g. Re, Rf, Rg1, Rg2, Rh1). Each gensenoside is different from the other according to the presence of different sugar moieties at different position of the dammarane. Not all ginsenosides possess the same biological activity. Some ginsenosides may act as antioxidants, prevent cancer or chemically induced tissues damage, and delay the process of aging. Others may be prooxidants, which can accelerate oxidative injuries. Glutathione is a major cytosolic protein which regulates the free radical levels by interconversion between the oxidized (GSSG) and the reduced (GSH) forms in the presence of enzymes including glutathione peroxidase (GPx), glutathione reductase (GR) and γ-glutamylcystenyl synthase (γ-GCS). It is also found that a change in GSH/GSSG is also related to cellular activity such as cell growth and cell death. In the present study, the effect of two ginsenosides, Rc (PPD) and Rf (PPT) were analyzed for their effect on glutathione metabolism in the C6 glioma cells. It was demonstrated that both Rd and Rf caused a dose-dependent increase in GSH/GSSG. However, their effect on the metabolizing enzymes: GPx, GR and GCS were different. Rd caused an increase in GR activity while Rf caused an increase in both GPx and GCS activities. It was concluded that ginsenosides enhance the glutathione redox status in the C6 glioma cells through activation of different enzymes.

1117 BREVETOXIN-INDUCED BRONCHOCONSTRICTION AND ANTAGONISM IS MODULATED BY THE ANTIPARALLEL CHARACTER OF SYNTHETIC SIDE CHAINS: CHARACTERISTICS FOR SUCCESSFUL PULMONARY DRUG DESIGN.

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Brevetoxins (PbTx) are potent polyether neurotoxic compounds produced by the dinoflagellate Karenia brevis. Inhalation of PbTx aerosolized by surf and wind induces nasal and/or trachebronchial irritation, and pM concentrations can produce asthma-like symptoms in humans. Last year we showed that beta-naphthoyl-brevetoxin (B-Nap), a synthetic derivative of the natural brevetoxin-3 binds with high affinity (Ki=1.2 nM) to the VSSC without increasing sodium flux, and antagonizes PbTx-induced bronchoconstriction in sheep. As the lead compound for investigating potential pulmonary therapeutics, B-Nap and a series of B-Nap analogs have been synthesized. Extensive conformational searches were used to select the most likely therapeutic candidates. Based on data published elsewhere, a sodium channel drug locus for B-Nap was postulated. Sodium channel function in whole-cell voltage clamped cultured neuro-2A cells (ATTC: CCL-133) was evaluated using the automated patch clamping device Port-a-Patch (Nanion Technologies). Consistent with our previous sheep bronchoconstriction work, analogs that displayed reduced sodium channel activity were the most active antagonists of brevetoxin action in voltage-clamped cells. In addition, those most active synthetic brevetoxin antagonists also are the most active in assays for cystic fibrosis. The computer conformational searches revealed that the most active B-Nap analogs possessed a structural feature whereby the side chain aromatic moiety lies back across the brevetoxin backbone, creating an antiparallel sandwiched H-K ring region. It is our hypothesis that the sandwich character is responsible for the potent antagonistic effects seen with B-Nap and to a lesser degree with several other analogs where the antiparallel character is less pronounced. Funded by P01 ES 10594.

1118 COMPARISON OF GENE EXPRESSION PROFILES IN LIVER OF RATS TREATED WITH RIDDELLIINE AND COMFREY.

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Comfrey (Symphytum officinale) is a perennial plant and has been consumed by humans as a vegetable, a tea and an herbal medicine for more than 2000 years. However, it is hepatotoxic and carcinogenic in experimental animals and hepatotoxic in humans. Pyrrolizidine alkaloids (PAs) exist in many plants and many of them cause liver toxicity and/or cancer in humans and experimental animals. In our previous study, we found that the mutagenicity of comfrey was associated with the PAs contained in the plant. Therefore, we suggest that carcinogenicity of comfrey result from those PAs. To confirm our hypothesis, we compared the expression of genes and processes of biological functions that were altered by comfrey (mixture of the plant with PAs) and riddelliine (a prototype of carcinogenic PA) in rat liver for carcinogenesis in this study. Groups of 6 Big Blue Fisher 344 rats were treated with riddelliine at 1 mg/kg body weight by gavage five times a week for 12 weeks or fed a diet containing 8% comfrey root for 12 weeks. Animals were sacrificed one day after the last treatment and the livers were isolated for gene expression analysis. The gene expressions were investigated using Applied Biosystems Rat Whole Genome Survey Microarrays and the biological functions were analyzed with Ingenuity Analysis Pathway software. Although there were large differences between the significant genes and between the biological processes that were altered by comfrey and riddelliine, there were a number of common genes and function processes that were related to carcinogenesis. There was a strong correlation between the two treatments for fold-change alterations in expression of drug metabolizing and cancer-related genes. Our results suggest that the carcinogenesis-related gene expression patterns resulting from the treatments of comfrey and riddelliine are very similar, and PAs contained in comfrey are the main active components responsible for carcinogenicity of the plant.

1119 RED TIDE TOXIN PRODUCES IN VITRO DEPOLARIZATIONS OF HUMAN AIRWAY SMOOTH MUSCLE.

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Florida red tides are occurrences resulting from overgrowth of the dinoflagellate *Karenia brevis*. This algae produces a number of potent polyether toxins collectively known as brevetoxins (PbTxs). Exposure to PbTxs produces an apparently reversible upper airways irritation and asthma-like symptoms in susceptible individuals exposed under natural conditions. Preparations of brevetoxins (PbTxs) were previously demonstrated to produce *in vitro* airway smooth muscle (ASM) contractions in canines, that were electrically-coupled to the depolarization (DP) of the ASM cell. This study describes the DP effects of PbTxs in *in vitro* ASM tissue obtained from normal and asthmatic humans and which demonstrates a greatly enhanced response in asthmatic preparations at the same level of exposure. Membrane potentials of *in vitro* ASM preparations were measured with a microelectrode before, during and after exposure to crude toxin (0.01-1.2 μg/ml) in both strip preparations (SPs) and cultured ASM reaggregate (RAs) preparations. The latter preparation results in the disruption of the normal peripheral nervous/ASM association. Exposure to PbTxs produced concentration-dependent DP in both non-asthmatic and asthmatic SPs. In the former, the responses did not occur in the presence of the blocking agents atropine or tetrodotoxin (TTX). In asthmatic SPs, atropine and TTX produced little effect, whereas verapamil blocked the PbTx-induced DP. The toxin was without effect in non-asthmatic cultured cells, whereas acetylcholine produced DP that was blocked in the presence of atropine, but not TTX or verapamil. This reaffirmed the presence of functional cholinergic receptors in cultured cells, and the PbTx-induced release of endogenous acetylcholine from remaining peripheral nerve endings in the *in vitro* ASM response. In contrast, the toxin produced significant DP in cultured asthmatic ASM cells, which were unaffected by either atropine or TTX but were blocked by verapamil. Different mechanisms for depolarization in response to PbTxs exposure may exist for normal and asthmatic or hyperreactive airways.

1120 BIPHASIC REGULATION OF *IL2* GENE EXPRESSION IN NAïVE CD4+ T CELLS: RATE LIMITING ROLES FOR TNF-α RECEPTOR SIGNALING AND CHROMATIN STRUCTURE.

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We describe a novel biphasic regulation of *Il2* gene induction in CD4+ T cells. Less than 10% of naïve CD4+ T cells rapidly (early phase) transcribe *Il2* during a period coinciding with peak IL-2 production in previously activated (Pre Ac) CD4+ T cells. *Il2* induction in most naïve CD4+ cells is delayed for at least 12–18 h (late phase). Our data suggest that a threshold level of c-Rel is critical for the induction of *Il2* in naïve and Pre-Ac CD4+ cells and show that this is provided by TNFR signaling during priming but not recall immune responses. We further suggest a model by which stable chromatin modifications within the proximal promoter of the *Il2* gene during priming and constitutive c-Rel abundance in Pre-Ac CD4+ T cells contribute to rapid *Il2* transcription in a secondary immune challenge.

1121 REGULATION OF TUMOR NECROSIS FACTOR-α AND INTERLEUKIN-1β-MEDIATED TNF RECEPTOR EXPRESSION AND PROINFLAMMATORY RESPONSES IN LUNG EPITHELIAL CELLS.

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The pleiotropic cytokines tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) mediate the pathogenesis of numerous inflammatory and fibrotic lung diseases. We previously demonstrated both of these molecules modify metallopro-

tease-dependent proteolytic cleavage of the two TNF receptors. However TNF-α or IL-1β-mediated regulation of cell surface expression, receptor activity and receptor-specific chemokine production are incompletely defined. Utilizing MLE-15 cells, we investigated alterations in both receptor solubilization via ELISA and the plasma membrane localization of TNF receptors and the metalloprotease TNF-α converting enzyme (TACE) using cell surface ELISAs after stimulation with TNF-α or IL-1β in the presence or absence of the metalloprotease inhibitor TAPI-2. In addition, we studied the effects of proteasome inhibition on constitutive and lig-and-induced receptor release as ectodomain shedding of cell surface proteins may involve proteasome activity. Finally, using receptor specific siRNA, we explored the role of TNFR1 vs. TNFR2 in the production of the inflammatory mediators macrophage inflammatory protein (MIP-2) and KC in order to further understand the effects of TNF-α and IL-1β-mediated TNF receptor regulation leading to inflammation in the context of pulmonary disease. In contrast to no effect of IL-1β, TNF-α or TAPI-2 increased membrane TNFR2 by two-fold compared to controls. Pretreatment with TAPI-2 prior to stimulation with either ligand resulted in enhanced cell surface TNFR2 (two to three-fold), suggesting that metalloprotease activity is involved in ligand-induced TNFR2 turnover. Proteasome inhibition altered shedding and cell surface expression without increasing cytotoxicity. Finally, TNF-α-induced MIP-2 and KC secretion was receptor-specific, indicating differential roles of the TNF receptors in TNF-α-mediated inflammatory responses.

1122 INHIBITORY EFFECT OF THE SAPONINS DERIVED FROM ROOTS OF PLATYCODON GRANDIFLORUM ON ANAPHYLACTIC REACTION AND MAST CELL ACTIVATION.

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Changkil (CK) is an aqueous extract of the root from 20-year-old Platycodon grandiflorum plants, prevented hypercholesterolemia, hyperlipidemia and hepatic fibrosis. CK saponin fraction (CKS) derived from CK had potent antioxidative effects such as superoxide radical scavenging activity and the inhibition of ROS production. Mast cells are key effector cells in the early phase allergic inflammation and in diverse immunological and pathological processes. In this study, we investigated the effects of CKS on anti-allergic effect on RBL-2H3 rat mast cells and on IgE-mediated passive cutaneous anaphylaxis (PCA) in mice. CKS reduced the anti-dinitrophenyl (DNP) IgE antibody-induced β-hexosaminidase and histamine release in RBL-2H3 cells. Also, the oral administration of CKS inhibited IgE-induced ear swelling was significantly reduced systemic passive cutaneous anaphylaxis reaction in mice. Taken together, these findings suggested that CKS has an anti-allergic activity and this might be useful for the clinical application to treat allergic diseases such as atopic dermatitis.

1123 *IN VIVO* AND *IN VITRO* MECHANISTIC ANALYSES OF LIPOPOLYSACCHARIDE (LPS)-INDUCED HEPATOTOXICITY DEMONSTRATE SIMILAR TOXICITY PATTERNS.

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Precision-cut liver slices are becoming accepted as an in vitro tool to study drug metabolism, xenobiotic-induced hepatotoxicity, and induction properties of drugs. To further verify its potential as a relevant in vitro model system, in vitro LPS data (precision-cut livers harvested after 6h, 1 day, and 2 days of treatment; at 10 μg/ml LPS and 50 μg/ml) were analyzed and compared to in vivo rat liver LPS data (6hr, 1 day, and 3 days at 2mg/kg and 8mg/kg), and analyzed in the context of a large reference rat toxicology database and a comprehensive set of pathway analysis tools. Strong agreement between the in vitro and the in vivo LPS response patterns was identified, with very similar early response patterns as determined by matches to validated biomarkers (signatures) for 'Hypoalbuminemia', 'Leukopenia', and 'Neutrophilia'. The analysis also revealed that after 1 day of either in vivo or in vitro treatment, the number and intensity of gene expression changes decreased dramatically, and returned to baseline values at 2 and 3 days. These analyses, as well as other methods to be described, reveal that significantly perturbed genes for both the 6 hr in vivo and 6 hr in vitro LPS treatments are associated with immune response, oxidative stress, and liver damage pathways, and also show common induction patterns for several signaling pathways including IL-10, IL-6, NF-kappa, and Toll-like receptor signaling. These data further validate the use and utility of the in vitro model system.

1124 SUPPRESSIVE EFFECTS OF PHORBOL-12-MYRISTATE-ACETATE ACTIVATED CYCLOOXYGENASE-2 EXPRESSION BY DIHYDROARTEMISININ IN MACROPHAGES.

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Artemisinin and its derivatives are renowned for their potent antimalarial activity. The water-soluble dihydroartemisinin, artemisinin derivative, is 4 to 5 times more active in vitro. In the present study, we investigated the effects of artemisinin and dihydroartemisinin on the cyclooxygenase-2 (COX-2) gene expression, which plays a crucial role in many physiological and pathological processes in macrophages. Treatment with dihydroartemisinin significantly decreased the production of PMA-induced prostaglandin E2 and the expression of COX-2 mRNA in PMA-activated RAW 264.7 macrophages in a dose-dependent manner. However, artemisinin did not affect PMA-induced prostaglandin E2 and the expression of COX-2 mRNA level. Transient transfection experiments also showed that PMA-induced increase in COX-2 promoter activities was suppressed by dihydroartemisinin. This study suggests that modulation of COX-2 expression by dihydro-artemisinin may be important in the prevention of inflammation.

1125 DEOXYNIVALENOL-INDUCED MAP KINASE ACTIVATION MEDIATED *VIA* THE RIBOSOME CAN INDUCE ROBUST PROINFLAMMATORY GENE EXPRESSION AND APOPTOSIS IN MONONUCLEAR PHAGOCYTES.

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Deoxynivalenol (DON), the most common trichothecene mycotoxin in cereal foods, causes several toxic responses, and thus adversely affects human and animal health. At the molecular level, DON inhibits translation and induces activation of mitogen activated protein kinases (MAPK) involved in cell survival or death via a poorly understood mechanism known as the "ribotoxic stress response". Here, we hypothesized that ribosomes mediate p38, JNK and ERK MAPK activation in the DON-induced ribotoxic stress response. We observed that uptake of radiolabeled DON in RAW 264.7 macrophages occurred within 15 min and was followed by robust p38, JNK and ERK MAPK activation 15 min later. Ribosomes were separated using a sucrose gradient and MAPK interactions in monosome and polysome fractions were assessed by immunoblotting. Notably, phosphorylated p38, JNK and ERK binding to the monosome was observed as early as 2.5 min, and was increased further in time-dependent manner. Interestingly, phosphorylated MAPK interaction was detectable in the polysome fraction at 5 min, suggesting a possible effect on translation. Using the fluorescence microscopy, phospho-p38 was localized in cytoplasm of DON-treated cells at 15 min and later moved to perinuclear region and nucleus. Taken together, these data suggest that the ribosome might function as a signal transducer and mediator for DON-induced MAPK activation with sequelae inflammatory gene expression and apoptosis as downstream.

1126 UNFOLDED PROTEIN RESPONSE INDUCED IN MACROPHAGES BY THE TRICHOTHECENE DEOXYNIVALENOL.

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Exposure of mice to the trichothecene deoxynivalenol (DON) induces expression of the proinflammatory cytokine interleukin-6 (IL-6) which drives aberrant IgA production and mimics human IgA nephropathy. Our lab has previously shown that, in murine EL-4 T cells, DON suppresses expression of Bip (glucose-regulated protein [GRP]-78), an ER chaperone that binds unfolded proteins in the ER lumen. Loss of Bip can trigger the unfolded protein response (UPR) and activate the UPR sensor ATF-6 and inositol-requiring enzyme (IRE)-1. IRE-1 has RNase activity that splice an intron in the RNA of X-box-binding protein 1 (XBP1) leading to transcription and translation of active XBP1. ATF6 and XBP1 are transcription factors that can bind cAMP-response elements (CRE) and drive expression of CRE-dependent genes such as IL-6. The purpose of this study was to examine the effects of DON on Bip and related up- and down-stream signal pathways in the macrophage. Bip protein expression was markedly decreased in mouse peritoneal macrophages following incubation with DON (500 ng/ml) for 1 h and was almost completely undetectable from 6 to 24 h. DON incubation was found to markedly

increase IRE-1, XBP-1 and ATF-6 protein expression within 1 h. Pretreatment of macrophages with an inhibitor of double-stranded RNA activated protein kinase (PKR) blocked expression of both IRE1 and IL-6. These data suggest that DON-induced IL-6 expression is PKR-dependent and might be mediated by Bip degradation and an ensuing UPR. (Supported by Public Health Service Grants DK58833)

1127 SUPPRESSION OF MAST CELL-MEDIATED ALLERGIC REACTION BY AMOMUM XANTHIODES.

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The mast cell-mediated immediate-type allergic reaction is involved in many allergic diseases such as asthma, allergic rhinitis and sinusitis. Stimulation of mast cells starts the process of degranulation resulting in release of mediators such as histamine and an array of inflammatory cytokines. In this report, we investigated the effect of aqueous extract of Amomum xanthiodes (Zingiberaceae) (AXE) on the mast cell-mediated allergy model and studied its possible mechanisms of action. AXE inhibited compound 48/80-induced systemic reactions and serum histamine release in mice. AXE decreased immunoglobulin E (IgE)-mediated passive cutaneous anaphylaxis reaction. AXE reduced histamine release and intracellular calcium from rat peritoneal mast cells activated by compound 48/80. Furthermore, AXE decreased the activation of p38 mitogen-activated protein kinase (MAPK) but not extracellular signal-regulated kinase and c-jun N-terminal kinase, and downstream tumor necrosis factor (TNF)-α production in phorbol 12-myristate 13-acetate and calcium ionophore A23187-stimulated human mast cells. Our findings provide evidence that AXE inhibits mast cell-derived allergic reactions, and that intracellular calcium, TNF-α, and p38 MAPK are involved in these effects.
This work was supported by Brain Korea 21.

1128 HIGH-THROUGHPUT SCREEN FOR SMALL MOLECULE HEDGEHOG SIGNALING INHIBITORS RELEVANT TO HUMAN EXPOSURE.

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The Hedgehog (Hh) signaling pathway plays an integral role in the patterning and development in the vertebrate embryo and aberrations in Hh signaling are associated with developmental defects including cleft lip/palate and cyclopia (holoprosencephaly or HPE). Postnatally, Hh signaling is postulated to play a pivotal role in healing and repair and inappropriate pathway activation is implicated in cancer. The Veratrum alkaloid cyclopamine is a potent inhibitor of Hh signaling and causes HPE-like defects in animal models. Human dietary alkaloids structurally related to cyclopamine were shown recently to be weak Hh signaling inhibitors. Additionally, screens have found the Hh pathway to be sensitive to inhibition by small molecules of diverse chemical structure unrelated to cyclopamine. Most Hh inhibitors antagonize the Hh signaling protein Smoothened (Smo), suggesting that it is innately sensitive to small molecule inhibition. Given the importance of Hh signaling in biological processes, the identification of pathway inhibitors relevant to human exposure may have important health implications. We screened small molecule libraries of marketed drugs, natural products, and pesticides for Hh signaling inhibition activity. Compounds were screened at 10μM using *Ptc*$^{-/-}$ mouse embryonic fibroblasts. These cells demonstrate ligand-independent Hh pathway activity which is sensitive to Smo antagonism. We identified five lead compounds (i.e., the dietary supplement, ipriflavone; the anti-fungal, tolnaftate; the antihistamine, carbetapentane; the monoamine oxidase type A inhibitor, clorgyline; and the hormone, β-estradiol) as putative Hh signaling antagonists. We determined the relative EC_{50} and EC_{90} for each compound and applying these concentrations found that they inhibited endogenous Hh target gene activation in a dose-dependent fashion.

1129 TRANILAST INHIBITS TRANSCRIPTIONAL ACTIVITY OF HYPOXIA INDUCIBLE FACTOR.

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Oxygen availability influences various physiological and pathological processes, such as inflammation, fibrosis, angiogenesis, and tumorigenesis. Cellular responses to hypoxic stress are primarily controlled by a heterodimeric transcription factor, hypoxia-inducible factor (HIF), which upon activation by either hypoxia or inhibition of HIF prolyl hydroxylases (PHD) induces expression of genes important for successful adaptation to low oxygen environment. Tranilast (RIZABEN®) is an anti allergic drug that has been approved for the treatment of bronchial asthma, allergic rhinitis, and atopic dermatitis in Japan, Korea, and China. Its potential use for the treatment of autoimmune diseases is currently under investigation. Several in vitro cell culture and in vivo studies have also revealed that tranilast may have other therapeutic benefits, such as antifibrotic, antiangiogenic, and antitumorigenic activity. In this study, we investigated whether the pharmacological activities of tranilast are mediated by its effect on the HIF signaling pathway. HIF-dependent transcriptional activity was significantly decreased by treatment of cells with tranilast. However, tranilast treatment had no effect on the level of the HIF-1α protein stabilized by either hypoxia or PHD inhibitors, indicating that inhibitory effect of tranilast on HIF activity is not due to the increased HIF-1α protein turnover. Furthermore, immunostaining of the HIF-1α protein in cells treated with tranilast showed that the majority of HIF-1α stabilized by PHD inhibition can translocate into the nucleus, excluding the possibility that tranilast blocks subcellular trafficking of HIF-1α protein. However, antifibrotic activity of tranilast does not appear to depend on HIF-1α, as the overall extracelluar matrix accumulation was similar in wild-type and HIF-1α deficient fibroblasts. Taken together, these results indicate that certain pharmacological activity of tranilast is selectively mediated at least in part by inhibition of HIF-dependent transcription.

1130 THE ROLE MAP3K1 IN NICKEL-INDUCED LUNG INJURY.

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Nickel is produced as an industrial byproduct. Inhalation of nickel leads to acute lung injury, characterized by disruption of the alveolus-capillary barrier. Injury triggers an immediate repairing process involving the activation of signal transduction pathways that regulate pulmonary epithelial cell migration and proliferation. Delayed injury repair leads to excessive leakage of fluids and inflammatory cells into the alveoli, thus impairing the normal functions of lung. The mitogen-activated protein kinase kinase kinase 1 (MAP3K1) is a member of the MAP3K family, involved in diverse pathophysiological processes. In vivo studies show that MAP3K1 is crucial for epidermal keratinocyte migration, a function required for re-epithelialization and closure of skin wounds. The objective of this study is to investigate the role of MAP3K1 in lung injury repair as a result of nickel exposure.
In this work, we used the *Map3k1*$^{\Delta KD/\Delta KD}$ mice, in which the promoter of *Map3k1*$^{\Delta KD}$ allele directed the expression of a kinase inactive MAP3K1 fused to β-Galactosidase. The fusion protein expression was detected by whole mount X-gal staining. In the lung of the *Map3k1*$^{\Delta KD/+}$ mice, X-gal positive staining was accumulated in the bronchial tree. Subsequent histological analyses located the X-gal positive cells in the bronchiole epithelial and smooth muscle cells. Western Blotting also detected the endogenous MAP3K1 protein in mouse lung tissues. To investigate whether MAP3K1 plays a role in nickel-induced lung injury, we continuously exposed the wild type and *Map3k1*$^{\Delta KD/+}$ and *Map3k1*$^{\Delta KD/\Delta KD}$ mice to nickel sulfate ($NiSO_4$) aerosol and measured survival rate. While nearly 50% wild type and *Map3k1*$^{\Delta KD/+}$ mice remained alive, only 12% *Map3k1*$^{\Delta KD/\Delta KD}$ mice survived at four days following nickel exposure. Based on these observations, we suggest that MEKK1 expression in bronchiole epithelial and smooth muscle cells may contribute to the protection against nickel-induced lung injury.

1131 PRETREATMENT WITH LOW CADMIUM CONCENTRATION AND THE ANTIOXIDANT ALPHA-LIPOIC ACID AFFORDS PROTECTION TO A HIGHER CADMIUM CONCENTRATION ON HEPG2 CELLS BY MODULATION OF THE ANTIOXIDANT AND INFLAMMATORY GENE EXPRESSION.

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Antioxidants are crucial for reducing oxidative stress and preventing cellular death. A mechanism for the observed antioxidant activity of alpha-lipoic acid (ALA) has been related to Nrf2 activation, a master transcription factor of antioxidant responsive genes. Cadmium (Cd) induces an antioxidant response by Nrf2 activation, however according to the dose, also evokes an inflammatory and apoptotic cellular expression. To study the mechanism of protection conferred by a low dose of Cd and compare it to the evoked by ALA pretreatment on exposure to a higher Cd concentration, we treated HepG2 cells to 1 μM Cd2+ or 5 mM ALA for 8 h followed by a exposure to 2.5 μM Cd2+ for 24 h. MTT-metabolic activity, Trolox-equivalent antioxidant capacity (TEAC) and key antioxidant and inflammatory quantitative gene expression were performed previous and 24h after 2.5 μM Cd exposure. 1 μM Cd showed no signs of toxicity at 8h post-exposure just as ALA pretreatment did. Exposure to 2.5 μM Cd were evidently toxic to cells and significantly augmented MTA1, HMOX1, TNFa, IL6, and CASP3 expression, however these responses were abrogated by ALA pretreatment. 2.5 μM Cd toxicity was importantly reduced by 1 μM Cd pretreatment with a concomitant reduction on HMOX1, TNFa, IL6 and CASP3 expression. Low dose exposure to Cd acts as an effective protective measure to higher toxic exposures. Down-regulation of

HMOX1 and inflammatory gene expression below a given threshold level could be the required element to maintain a healthy condition of a cell exposed to toxic agents.

1132 VALIDATION OF INHININ B ELISA.

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Inhibin is a heterodimeric glycoprotein consisting of an alpha and a beta subunit linked by disulphide bridges. While the alpha subunit remains constant the different inhibins are defined by the beta subunit -currently A and B. Inhibin is produced by the testes as well as the ovaries, and is responsible for the selective negative feedback control of FSH secretion. In males, inhibin B is synthesized by the sertoli cells in the testis, and can be used as a direct marker of sertoli cell function and spermatogenesis in adult males. Hence, it is considered a biomarker for detecting testicular damage. Here, we report the validation of the human inhibin B ELISA from DSLabs to measure male rat serum inhibin B levels.
Validation criteria included the following: linearity, reproducibility, recovery, kit lot to lot variability, and stability of inhibin B over multiple freeze/thaw cycles. Our results showed very strong linearity for both samples that were spiked with calibrators from the ELISA kit, and with castrated rat serum that have been diluted with different amounts of intact rat serum (r^2 >0.9). Recovery of spiked samples (62-79%) was not as good as the recovery from castrated/intact serum dilutions (76-100%). Although lot to lot variability (% CV >200%) was high, kit to kit variability from the same lot was good (% CV <8%). Inhibin B levels showed good stability over multiple freeze/thaw cycles (%CV <7%). Using this kit, biologically relevant changes in inhibin B levels in castrated, hypophysectomized and intact animals were detected. We conclude that this human inhibin B ELISA kit can detect changes in the levels of inhibin B in rat serum.

1133 OXIDATIVE STRESS DAMAGE CAUSED BY FLUORIDE EXPOSURE DECREASED THE IN VITRO FERTILIZATION IN RAT SPERMATOZOA.

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The major sources of exposure to fluoride are drinking water, dental products, food, and pesticides. High fluoride concentration in groundwater has been reported in many places as up to 30 mg/l. Fluoride exposure has been associated to oxidative stress in soft tissues as renal, liver and testes. Recent advances have been implicated the oxidative stress as one of the major causative factor for male infertility, due to sperm cells are susceptible to damage oxidative by reactive oxygen species. The aim of this study was to evaluate the oxidative stress role caused by fluoride exposure in Wistar male rats on *in vitro* fertilization capacity of spermatozoa. Rats were administered with NaF at dose equivalent to 5mg fluoride/kg/day or deionized water for control group by gavage during 60 days. After fluoride exposure, plasmatic level of fluoride in rats was 0.26 ± 0.03 ng/ml, which is compatible with chronic environmental or occupational exposure to fluoride in humans. We evaluated in spermatozoa: i) the superoxide dismutase (SOD) activity, ii) the generation of superoxide anion, iii) thiobarbituric acid reactive substances (TBARS) concentration, and iv) *in vitro* fertilization assay. Sperm of rats exposed to fluoride showed a significant decrease of SOD activity (~33%) accompanied with significant increase in the generation of superoxide anion (~40%), and significant increase in TBARS concentration (~50%), relative to the control group. The percentage of eggs fertilized with sperm of rat exposure to fluoride was significantly lower (13%) than control group (71%). Oxidatively damaged spermatozoa are characterized by the accumulation of lipid hydroperoxides in the plasma membrane, thus integrity and fluidity of the sperm plasma membrane is able to decrease the fertilization capability of spermatozoa.
The subchronic exposure to fluoride in relevant biological doses caused oxidative stress damage in spermatozoa, declining the ability of sperm to fertilize oocytes *in vitro.*

1134 DETERMINATION OF PEAK HORMONE LEVELS FOLLOWING A SINGLE INTRAMUSCULAR INJECTION OF HUMAN CHORIONIC GONADOTROPHIN (HCG) IN DOGS.

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Testicular steroidogenesis can be induced by an acute administration of human chorionic gonadotrophin (hCG). However, detailed studies on testicular steroid response and time course of the response in dogs have not been previously described. The present study characterizes alterations in androgen production following stimulation with hCG. Serum testosterone, dihydrotestosterone (DHT), and luteinizing hormone (LH) levels were measured following a single intramuscular (IM) injection of human chorionic gonadotropin (hCG) in dogs (100 IU/kg). The study was conducted in two phases to identify a rough peak of serum hormone levels in Phase 1, followed by Phase 2, with focused sampling at the highest expected serum hormone levels. Beagle dogs received injection of hCG on Day 1 (Phase 1) and Day 16 (Phase 2) of the study. During Phase 1 of the study, blood samples were collected from all animals hourly, up to 12 hours and 24 hours both prior to and following hCG injection. During Dosing Phase 1, the highest mean levels of testosterone and DHT were seen at 24 hours after hCG injection. For LH, the highest level was seen approximately 2 hours after hCG injection. Based on the results from Phase 1, during Phase 2 of the study, blood samples were collected from all animals, at 30 minutes, 2, 6, 8, 12, 16, 24, 36, and 48 hours both prior to and following hCG injection. Although the hormone levels at each time point were variable, the highest mean levels and time to peak following hCG injection were generally similar to those seen in Phase 1. The only difference was for testosterone, where the highest mean level during Phase 2 occurred approximately 16 hours after hCG injection versus 24 hours in phase 1. By 48 hours after injection, the levels of all three hormones had returned to baseline. The results of this study confirm that a 100 IU/kg intramuscular injection of hCG in the dog caused an increase in the serum levels of testosterone, DHT, and LH, which were maximal at approximately 16, 24, and 2 hours, respectively.

1135 EFFECTS OF RESTRICTED FEEDING ON PREGNANCY OUTCOME AND BLOOD PARAMETERS IN RABBITS.

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We examined the relationship between the duration of restricted feeding and the occurrence of abortion (Exp. 1) and the changes in progesterone concentration and lipid- and coagulation-related parameters prior to the occurrence of abortion (Exp. 2).
Exp. 1 was composed of 5 groups of Kbl:NZW pregnant rabbits (8 animals/group). They were subjected to restricted feeding as follows; free access to food (A), 60g/day between GD 6 and 18 (B), 20g/day between GD 6 and 18 (C), GD 19 and 28 (D), and GD 6 and 28 (E). Exp. 2 was composed of 2 groups of Kbl:NZW pregnant rabbits; 12 animals allowed to free access to food (N), 22 animals given 20g/day between GD 6 and 28 (RC, 12 animals had Caesarean section on GD 28; RA, 10 animals aborted between GD 23 and 27). Hematological and blood chemical examinations were performed on GD 6, 22 and 28. Live fetuses were counted on GD 28 and observed for morphological development.
In Exp. 1, abortion occurred only in the D (2/8) and E (8/8). The incidence of fetal lethality increased only in the B and C. A slight inhibition of fetal growth was recorded in the D. There were no morphological abnormalities in the fetuses. In Exp. 2, lower fibrinogen, platelets and Antithrombin III, shortening of APTT (GD 22 and 28), prolongation of PT and higher cholesterol (GD 22) were observed in the RC compared with the N. In the RA, values of platelets, Antithrombin III, PT and progesterone concentration on GD22 were significantly different from those in the RC. Especially, individual progesterone concentration were lower (5 ng/ml or less) in the RA than in the RC.
Restricted feeding during the period of organogenesis induced an increase in fetal lethality and that during the later period of gestation induced abortion. Coagulation-related parameters showed more prominent changes in the RA than in the RC and the progesterone concentration was lower in the RA than in the RC prior to the occurrence of abortion. This suggests that decreased progesterone levels prior to the abortion have a close relation to the occurrence of abortion in pregnant rabbits subjected to the restricted feeding.

1136 POSTNATAL DEVELOPMENT AND EVALUATION OF THE IMMUNE SYSTEM IN THE CYNOMOLGUS MONKEY MODEL (MACACA FASCICULARIS).

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Cynomolgus monkeys are frequently used during pre-/postnatal toxicity evaluation of biopharmaceuticals. Neither the pediatric nor the ICH S8 immunotoxicity guideline provide direct guidance for the evaluation of developmental immunotoxicity during infancy. Whereas the embryonic/fetal development of the immune system has recently been described (Buse 2005, J Immunotoxicol 2:211) such information is scarce for the postnatal development in this species. The present work aims at the postnatal development of the immune system in cynomolgus monkeys. Immune system evaluation comprised the following parameters: Blood immunophenotyping (IPT) using CD3, CD4, CD8, CD20 and CD16 antibodies (n = 26-32 infants), T cell-dependent antibody response (TDAR, n = 5-6 infants) using keyhole-limpet-hemocyanin (KLH), lymphocyte proliferation (n = 3 infants

and n = 3 adults) and natural killer (NK)-cell activity (NKTEST®, Orpegen-Pharma, Heidelberg, Germany) in 3 infants and 3 adults. Infant animals were one and 24 months old and adult animals were older than six years. Blood IPT revealed cell numbers within the adult reference ranges for all lymphatic subsets by one months of age with NK cells being consistently in the lower range. The CD4:CD8 ratio decreased from 2.2 at one month to 1.3-.1.6 at 12 months of age. Compatible with the IPT data, infant NK cell activity at 12-25 months was lower compared to adult (> 6 years of age) animals and the response to the functional stimulator IL-2 was reduced in the infants. In contrast, lymphocyte proliferation in 15-18 months old infants was comparable to that of adult animals with or without the presence of IL-2 based upon thymidine incorporation. TDAR responses (functional antigen presenting cells, T-cells, and B-cells and antibody-producing plasma cells) were evident by 3 months of age and a pronounced secondary antibody response to a booster immunization approximately 3 months thereafter. Overall, these data demonstrate general functionality of the cynomolgus monkey immune system in early postnatal life.

1137 THE EFFECTS OF *IN VIVO* EXPOSURE OF METHOXYCHLOR TO IMMATURE RATS ON SERUM PROGESTERONE AND ESTRADIOL LEVELS AND THE *EX VIVO* FORMATION OF PROGESTERONE BY THECA-INTERSTITIAL CELLS.

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Sponsor: V. Castranova.

Exposure to the pesticide methoxychlor (MC) in rodents is linked to impaired steroid production, ovarian atrophy and reduced fertility. Effects of in vivo MC treatment are thought to be mediated mainly by the active metabolite, HPTE. Previous in vitro studies on cultured ovarian cells have reported that HPTE inhibited P450 cholesterol side-chain cleavage activity resulting in decreased progesterone (P) and estradiol (E) production, suggesting direct ovarian effects. The current studies examine whether in vivo exposure to MC (0, 20-200 mg/kg) for 5-6 days alters serum P or E levels or ex vivo production of P by theca-interstitial cells from immature female rats primed with or without pregnant-mare serum gonadotropin (PMSG). In animals not treated with PMSG, serum P levels declined progressively with the dose of MC administered starting at the 100 mg/kg dose (43% of control) to 30 % of control at the 200 mg/kg dose. In the studies where animals were exposed to MC and to PMSG during the last 2 days of MC exposure, serum P was no different than the control level (4.97 ± 0.58 ng/ml in control animals); however, serum E levels declined to ~72 % of control. In ex vivo studies on ovarian cells obtained from animals exposed in vivo to MC but without PMSG, P production under basal conditions of cells exposed to MC was no different than control; however, when cells were exposed to hCG, cells exposed in vivo to MC produced 2.1-fold more P than control cells. In ex vivo studies on ovarian cells obtained from animals exposed in vivo to MC but also to PMSG, ovarian cells incubated under basal conditions and exposed to MC produced 1.5-fold more P than control cells. These studies demonstrate that the pattern of circulating P or E and ovarian cell production of steroids in response to in vivo exposure to MC is influenced by the maturational status of ovarian follicles and to possible differences in how female rats metabolize MC and /or its major steroid products.

1138 ANALYSIS OF CYTOGENETIC AND DEVELOPMENTAL EFFECTS ON PRE-IMPLANTATION MOUSE EMBRYOS AFTER MATERNAL EXPOSURE TO TRICHLORFON.

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Presently, we assessed cytogenetic and developmental toxicity in 3-day mouse embryos following maternal exposure to trichlorfon by drinking water at low levels (2, 10, or 50 mg/kg/day) for one month before pregnancy. Then the females were paired with males overnight. The pregnant mice were administered continuously until they were killed on the day of gestation 3. On day 3 of gestation, blastocysts were collected and evaluated for gross morphology, micronuclei (MN) frequency, cell number, fragmented nuclei and pycnotic nuclei. Trichlorfon exposure neither affected mean MN number and MN frequency in pre-implantation mouse embryos, nor maternal weight gain, implantation rates, or reproductive success (P>0.05). A significant decrease in the embryo cell number of pre-implantation embryos indicating developmental damage was observed in the 50mg/kg trichlorfon treatment group in comparison to control but not in 2 and 10 mg/kg groups. There were also a significant decrease in the blastocysts proportion, and a significant increase in morula proportion of pre-implantation embryos in the 50mg/kg group. Therefore, trichlorfon exposure under the present doses did not affect maternal weight gain and reproductive success, nor where cytogenetic toxicity evident in pre-implantation embryos. The lack of effects of trichlorfon on any in vivo reproductive and fetal endpoints suggests that, for trichlorfon, a hazard of reproductive toxicity below 50 mg/kg/day (which includes the highest trichlorfon residue levels detected from vegetables in China) is not expected. However, a simultaneous decrease in the cell number, blastocysts proportion and increase in morula proportion in 50 mg/kg group may reflect an embryonic developmental disadvantage resulting from maternal treatment with trichlorfon.

1139 CONSEQUENCES OF PRENATAL PFOA EXPOSURE ON MOUSE MAMMARY GLAND GROWTH AND DEVELOPMENT IN F1 AND F2 OFFSPRING.

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Perfluorooctanoic acid (PFOA) is a known developmental toxicant with ubiquitous presence in industrial applications and the ambient environment. We previously reported that prenatal PFOA exposure results in delayed development of the mouse mammary gland (MG) in F1 female offspring. To determine consequences of this delayed MG development on lactational function and subsequent development of F2 offspring, F1 females exposed transplacentally to 0, 1 or 5 mg PFOA/kg/day (control, 1P, 5P; gestation days 1-17), were bred to generate F2 offspring (no direct exposure to PFOA). F2 offspring were monitored for growth and development from postnatal day (PND) 1-22, and F1 dam MG function was assessed on PND10 by lactational challenge. MG tissue was isolated from both F1 and F2 females at necropsy on PND10 and 22, and scored for age-appropriate development on a 1-4 scale. As hypothesized, MG morphological scores were lower (p<0.05) in 1P and 5P F1 dams evaluated on PND10, and in 5P F1 dams on PND22. However, no effect of treatment on milk production (volume after 30-min nursing) or maternal behavior (time to initiate nursing) was detected on PND10. Body weight of F2 pups was similar between groups on PND1-10, however, starting on PND14 and persisting through PND22 body weight of 1P F2 offspring was significantly higher than controls. MG developmental scores in F2 pups were similar to control at PND10, but lower among P5 F2 offspring on PND22. The time of eye-opening was similar in all groups. These findings confirm previous PFOA-induced delays in lactating mammary gland differentiation, with a current LOEL for these effects at 1 mg/kg/d, and suggest that these delays have little, if any, deleterious effects on the F2 offspring early in life; further evaluation of F2 offspring will illuminate whether adverse health effects may result in adult life. (This abstract does not necessarily reflect EPA policy; SSW funded by EPA CR833237, NIH T32 ES007126.)

1140 NEONATAL EXPOSURE TO DES INDUCES DOSE-DEPENDENT DELAYED EFFECTS AT DOSES SHOWING ESTROGENIC ACTIVITY IN FEMALE DONRYU RATS.

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The neonatal exposure to endocrine disrupting chemicals (EDCs) with estrogenic activity is known to induce irreversible damage to hypothalamo-pituitary-gonadal axis called as androgenization in females, which immediately disrupts reproductive organ development before puberty. Recently, delayed effects of the neonatal exposure to estrogenic EDCs, which are inducible by lower treatment, have been focused as another type of neonatal effects, because these effects can't be detected in short-term bioassays. This type has been already reported as 'delayed anovulatory syndrome' (DAS), but its disruption pathways and endpoint markers remain fully undetermined. We investigated the relationship between occurrence of delayed effects and dose dependency in long-term reproductive organ responses to neonatal exposure to diethylstilbestrol (DES) in female rats. Female Donryu rats treated with single dose of DES at 0.15 to 1500 ug/kg body weight within 24 hours after birth were observed for 12 months. In addition, the rats were initiated with ENNG at 10 weeks of age to investigate uterine carcinogenesis. Typical androgenization was observed at 1500 ug/kg and 150ug/kg before puberty. Although no changes were detected before puberty, early onset of persistent estrus was detected in rats at 1.5 ug/kg body weight (at 21 weeks of age) and higher. Uterine adenocarcinoma development was promoted at 150 ug/kg. In uterotrophic assay, single dose treatments with DES at 1.5 ug/kg and higher exerted estrogenic activity. These results indicate that delayed effects were induced by neonatal exposure to DES at the doses showing estrogenic activity with dose-dependent manner. Estrous cyclicity is a useful

marker to detect the effects. In addition, prolonged persistent estrus reflecting higher relative E2 levels might play an important role in promoting effects on uterine carcinogenesis.

1141 OVARIAN FOLLICLE COUNTING USING PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA) AND SEMI-AUTOMATED IMAGE ANALYSIS IN SPRAGUE DAWLEY RATS.

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Ovarian follicle counting is used as a method to assess ovarian toxicity in reproductive toxicity studies. Traditionally, ovarian follicle counting has been performed on hematoxylin/eosin (H&E) stained sections; however, the use of immunohistochemical methods including human cytochrome P450 1B1 (CYP1B1) and proliferating cell nuclear antigen (PCNA) have been explored. In this study, both ovaries from ten 3-month-old female Sprague Dawley rats were formalin-fixed, trimmed, paraffin-embedded, and sectioned at 5 µm thickness. Serial sections were stained using routine H&E and immunohistochemistry for PCNA at five 100 µm intervals for a total of five sections per ovary per stain. Ovarian primordial follicle counting was performed independently by two pathologists and two technicians and follicle counts from the H&E and PCNA stained sections were compared. Semi-automated image analysis of PCNA stained sections was also performed and results were compared to manual follicle counts. This study demonstrated that PCNA immunohistochemistry is a valid method to enhance detection of ovarian primordial follicles with decreased variability in follicle counts between observers compared to the use of H&E staining. In addition, semi-automated image analysis of PCNA stained sections can be used to accurately perform automated ovarian follicle counts.

1142 TRIMETHYL NONANOL: A COMBINED REPEATED DOSE TOXICITY STUDY WITH THE REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN CRL:CD(SD) RATS.

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Trimethyl nonanol is an intermediate used in the production of ethoxylated surfactants. Groups of 12 male and 12 female Crl:CD(SD) rats were administered trimethyl nonanol daily, by oral gavage at dose levels of 0 (control), 20, 100, or 500 mg/kg/day. Females were dosed once daily for two weeks prior to breeding, during breeding (up to two weeks), gestation (three weeks), and lactation through postpartum day 4. Females were necropsied on post-partum day 5. Males were dosed two weeks prior to breeding and continuing through breeding until necropsy (test day 34). Administration of 100 or 500 mg/kg/day trimethyl nonanol resulted in parental systemic toxicity. The primary effect appeared to be an increase in absolute and relative liver weights and corresponding hepatocellular hypertrophy in males given 100 or 500 mg/kg/day and in females given 500 mg/kg/day. Treatment-related decreases in T3, T4 and increases in TSH concentrations were noted in the same groups, and were accompanied by increased thyroid weights in high-dose males and females and slight hypertrophy of follicular epithelial cells in males given 500 mg/kg/day. The thyroid changes may have been a consequence of hepatic enzyme induction leading to enhanced thyroid hormone metabolism, as this phenomenon has been demonstrated for a wide range of hepatic enzyme inducers. Males given 500 mg/kg/day also had increased kidney weights that corresponded with very slight or slight multifocal degeneration of renal tubules. Treatment-related hyaline droplet formation was noted in male rats given 100 or 500 mg/kg/day. There were no adverse effects of trimethyl nonanol on neurological or reproductive function. The no-observed-effect level (NOEL) for general toxicity was 20 mg/kg/day. The NOEL for neurological and reproductive effects was 500 mg/kg/day, the highest dose level tested.

1143 ORAL BIOAVAILABILITY AND KINETICS OF BENZO(A)PYRENE DISPOSITION IN OVARIAN TISSUES OF F-344 RATS.

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The process of reproduction in mammals is highly susceptible to environmental toxicants such as benzo(a)pyrene (BaP). Studies have shown that BaP bioavailability varies with the route of exposure and the bioavailable dose in tissues impact the events that lead to toxicity. The objective for undertaking this research is to investigate whether tissue DNA acts as the internal trapping agent for BaP reactive metabolites present in liver and reproductive tissues. Towards this end, concentrations of BaP metabolites and BaP-DNA adducts from these tissues were measured. Doses of 0 and 5 mg/kg BaP, representing control and exposure dose respectively were administered to F-344 female rats through oral gavage for 28 days. After 1, 7, 14 and 21 days post-BaP exposure, rats were sacrificed; blood, liver and ovary tissues were removed. Benzo(a)pyrene/metabolites from plasma and tissues were extracted using a liquid-liquid (water, methanol, and chloroform) extraction method and analyzed by a reverse phase HPLC method. DNA was isolated and analyzed for BaP-DNA adducts by the ^{32}P-postlabeling method using a four-directional TLC system. The concentrations of BaP total metabolites and reactive metabolites such as the 7, 8-dihydrodiol, and 3,6-quinone in plasma, ovary, and liver showed a gradual decrease from day 1 to day 21 post BaP administration. Exposed ovaries showed the highest DNA adducts levels compared to liver ($P < 0.05$). However, with increase in post-cessation of exposure, the adduct levels in liver tissue started declining rapidly, from day 1 to day 21. The adduct levels in ovary showed an insignificant decline from day 1 to day 7 and showed a significant decline thereafter. A concordance between BaP reactive metabolite, and adduct concentrations indicate that the bioavailability of reactive metabolites determine the binding with DNA and consequently the formation and persistence of adducts (funded by the NIH grants G12 RR03032, 1S11ES014156-01A1, 1RO3CA130112-01, 1U54HD0431501-09 and RO1 HD020419-19S1).

1144 MODULATION OF INTRATESTICULAR FUNCTION IN F-344 RATS BY BENZO(A)PYRENE.

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The objective of this study was to evaluate the reproductive risk associated with exposure of male F-344 rats to inhaled benzo(a)pyrene (BaP), an environmental toxicant. Treatment consisted of sub-chronic inhaled BaP (75µg BaP/m^3), 4 hours daily for 60 d, while controls were unexposed(UNC). Blood samples were collected immediately after the cessation of exposures(time 0)and at 24,48,and 72h,to assess the effect of bioavailable BaP on plasma testosterone and luteinizing hormone (LH) concentrations by RIA. Rats were sacrificed after the last blood collection following which, testes were harvested and weighed. The left testes were fixed for histology and morphometric analyses while the right testes were used for the quantitation of sonication-resistant spermatid nuclei and intra-testicular testosterone (ITT) and estradiol 17-β (E_2). BaP exposure reduced testis weights compared with UNC (2.01 ± 0.11 vs. 3.04 ± 0.16 g; $P < 0.025$), and total weight of tubules and total tubular length per paired testes were reduced 27% and 39%, respectively in BaP-exposed rats ($P < 0.01$) compared with UNC rats. The number of sonication-resistant spermatids was significantly reduced in BaP-exposed versus UNC rats. BaP exposure significantly decreased plasma testosterone and ITT and E_2 concentrations compared with those of UNC rats. The decrease in circulating plasma testosterone was accompanied by a concomitant increase in plasma LH concentrations in BaP-exposed versus control rats ($P < 0.05$). These data suggest that sub-chronic exposure to BaP contribute to reduced intratesticular testosterone and spermatogenesis(funded by the USPHS grants, G12RRO3032, U50ATU3989-48-06, 1U54HD0431501-09, RO1 HD020419-19S1, S11ES014156-01, and 1RO3CA130112-01).

1145 EVALUATION OF OVOTOXICITY INDUCED BY 7, 12-DIMETHYLBENZ[A]ANTHRACENE AND ITS 3, 4-DIOL METABOLITE UTILIZING A RAT *IN VITRO* OVARIAN CULTURE SYSTEM.

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The polycyclic aromatic hydrocarbon, 7,12-dimethylbenz[a]anthracene (DMBA), destroys ovarian follicles. Disruption of follicles by DMBA is attributed to the bioactivation of DMBA to a 3,4-diol-1,2-epoxide metabolite via cytochrome P450 enzymes and microsomal epoxide hydrolase (mEH). In in vitro cultures of neonatal rat ovaries incubation with DMBA results in follicle loss, suggesting ovaries bioactivate DMBA to its ovotoxic form. DMBA-3,4-diol (3,4-diol) is one of the intermediates in the DMBA metabolic pathway and is generated by mEH. The present study was designed to compare the ovotoxicity of 3,4-diol and DMBA and evaluate the role of mEH in ovarian culture. Ovaries from postnatal day 4 (PND4) Fischer 344 rats were incubated with control medium (DMSO) or 3,4-diol (12.5 nM – 1 µM) for 15d; or with control medium, 2 mM cyclohexene oxide (CHO, mEH inhibitor), 1 µM DMBA or 75 nM 3,4-diol, or 1 µM DMBA or 75 nM 3,4-diol and

2mM CHO for 6 days. After incubation, ovaries were prepared for histological evaluation by classification and counting of follicles. Following 15d incubation, compared to control, 3,4-diol reduced ($p < 0.05$) primordial (≧ 12.5 nM) and primary (≧ 75 nM) follicles. All healthy follicles were depleted by 3,4-diol at ≧ 250 nM. These concentrations of 3,4-diol are lower than those that cause similar damage with DMBA, thus the intermediate 3,4-diol is more ovotoxic than DMBA. Whereas, CHO prevented DMBA-induced follicle loss, follicle loss caused by 3,4-diol was not affected by CHO. These findings demonstrate that mEH is required for the bioactivation of DMBA by the rat neonatal ovary and that bioactivation most likely involves formation of the intermediated metabolite, 3,4-diol. (ES09246 and NIEHS Center Grant 06694).

1146 CIGARETTE SMOKE AND BENZO(A)PYRENE CAUSE FOLLICLE LOSS *IN VIVO* AND *IN VITRO* AT PHYSIOLOGICALLY RELEVANT CONCENTRATIONS.

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Cigarette smoke (CS) is a documented reproductive toxicant that has been linked to impaired fertility and an earlier onset of menopause. Benzo(a)pyrene (BaP) is a polycyclic aromatic hydrocarbon that is present in CS and at high concentrations is an ovarian toxicant. The goal of the current project was two-fold: to determine the underlying mechanisms mediating the adverse effects of smoking on ovarian function; and second, to determine the effects of BaP in concentrations representative of those in human follicular fluid on follicle loss. Female C57BL/6 mice, 8-9 weeks old, were exposed to CS using a Shapiro chamber for 8 weeks, euthanized and their ovaries excised and employed for histology and immunohistochemistry (IHC) or Western blot analysis. Ovaries were serially sectioned and follicle counts conducted on every 10th section. There were significantly fewer primordial and transitional follicles in smoke exposed mice than age-matched controls. The percentage of follicles recruited into the growing pool was significantly higher in smoke exposed compared to the control group. Using markers for apoptosis, IHC analysis indicated that Fas expression was unchanged between the groups; however, expression of BMP15, an oocyte-specific protein required for growth, was expressed in lower quantities in smoke exposed mice. Western blot confirmed these findings. To determine the effect of BaP on follicle loss 4 day old mouse ovaries were treated with BaP (0-10,000 ng/ml) in MS752/1 media for 6-48h followed by 24h recovery. Bcl-2 protein expression was decreased in mice treated with 10 ng/ml for as little as 6h whereas total Bax expression went unchanged. The Bax:Bcl-2, a ratio thought to determine if apoptosis will occur, was altered to favour apoptosis. Taken together these results suggest that CS- and BaP-induced follicle loss may be mediated via decreased growth support for the follicle and activation of the Bax pathway and apoptosis. Consequently, accelerated ovarian failure due to smoke exposure could potentially be impeded by targeting downstream members of the cascade it initiates.

1147 GLUCOCORTICOID RECEPTOR ACTIVATION RESTRAINS THE GROWTH-PROMOTING EFFECT OF TCDD ON MAMMARY EPITHELIAL CELLS.

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TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin), a high affinity ligand for the aryl hydrocarbon receptor (AhR), is a known human carcinogen and has effects on cell cycle progression and apoptosis. It has been reported that AhR activation by TCDD can stimulate proliferation of normal mammary epithelial cells and induce tumorigenic transformation. We evaluated the effect of AhR activation on proliferation of SCp2 cells, a functionally normal mouse mammary epithelial cell line, and show that TCDD treatment dose-responsively increases proliferation when the cells are grown in serum free media supplemented with hydrocortisone (HC), insulin and prolactin. Given that abnormal cell proliferation is an important step for tumorigenic progression, we next evaluated whether increased proliferation was associated with transformation by assessing whether TCDD-treated SCp2 cells form colonies in soft agar. There was no colony formation in either TCDD or vehicle treatment groups, suggesting that TCDD did not transform the SCp2 cells. Our next studies were conducted to explore the mechanism of increased proliferation. The glucocorticoid receptor (GR) signaling pathway has been reported to cross talk with the AhR pathway to alter functional activity of other types of epithelial cells. Therefore, we hypothesized that the presence of HC in the media was influencing the proliferation of the TCDD-treated cells. Interestingly, when we withdrew hydrocortisone from the media, TCDD's growth-stimulating effects were even more robust. Specifically, with HC (1ug/ml) in the media, TCDD (1 nM) increased the cell proliferation 2-fold, whereas in the absence of HC the increase was greater than 4-fold. Similar effects were seen when RU486 (Mifepristone) was used to block GR activation. These data add to the growing, and sometimes divergent, body of literature seeking to elucidate the mechanism and biological significance of the interaction between the AhR and GR signaling pathways.

1148 EFFECTS OF MOTORCYCLE EXHAUST AND COTREATMENT WITH VITAMIN E ON SPERM CONTENT AND INTERLEUKIN-6 EXPRESSION IN RAT TESTIS.

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The motorcycles (scooters) equipped with 2-stroke engines are a popular means of transportation in many parts of the world. In addition to motorcycles, the 2-stroke engine is used in a variety of applications including outboard boats, snowmobiles, lawn mowers, and trimmers. The 2-stroke motorcycle exhaust (ME) contains benzo(a)pyrene, 1,3-butadiene, and many toxic chemicals. The major objectives of the present study were to determine the effects of ME on sperm content and inflammatory cytokine expression in rat testis and the ability of vitamin E to protect rats from the ME effects. Male rats were exposed to 1:10 diluted ME 2 hr daily with or without cotreatment with 50 mg/kg vitamin E p.o. once daily, Monday through Friday, for 4 weeks. ME inhalation produced a 52% decrease of sperm content in the testis. In contrast, ME and vitamin E cotreatment did not cause significant changes of the sperm content. The results of real-time RT-PCR analysis showed that ME resulted in 2- to 3-fold increases of interleukins IL-6 and IL-1β mRNA expression in the testis and cotreatment with ME and vitamin E blocked the ME-mediated increases. The results of ELISA analysis showed that ME increased testicular IL-6 protein level and ME-vitamin E cotreatment reduced the increase mediated by ME. The present study demonstrates that ME inhalation decreases sperm content and increases IL-6 mRNA and protein expression in rat testis. The protective effect of vitamin E against ME toxicity suggests that oxidative stress is a possible mechanism of toxicity.

1149 DOES BUTYL ISOCYANATE PLAY A ROLE IN THE TUBULIN DEPOLYMERIZING ACTION OF THE FUNGICIDE BENOMYL?

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The fungicide benomyl is a testicular toxicant that adversely effects the testis by depolymerizing Sertoli cell microtubules with subsequent germ cell sloughing. Previous studies have implicated the benomyl metabolite carbendazim as the active agent in microtubule depolymerization. However, when benomyl is broken down to carbendazim, butyl isocyanate (BIC) is also a product. BIC is chemically reactive per se with a short half life (approx 1 minute) in aqueous media. In the present study the contribution of BIC to the effect of benomyl on microtubule polymerization has been investigated. Additional experiments sought to identify protein sites modified in the presence of BIC. In vitro studies were conducted with bovine brain tubulin (Cytoskeleton). Benomyl inhibited the rate of tubulin polymerization with an IC50 of 27.3uM. As predicted from earlier studies, carbendazim was the more potent inhibitor of polymerization with an IC50 of 14.95uM. The IC50 for BIC was 90uM. Because of BIC's reactivity, the time course and concentration of BIC accessing tubulin would differ after bolus addition to aqueous media vs continuous formation with benomyl breakdown. The fluorescent probe 1-anilino-8-naphthalene sulfonate was used to detect protein conformational changes after pretreatment with either benomyl, carbendazim or BIC at concentrations from 10-400uM. ANS fluorescence was unaltered by addition of carbendazim but markedly increased in a dose dependent manner by prior treatment with either benomyl or BIC. The interaction of benomyl or it's metabolites with tubulin did not appear to involve protein sulfhydryls as measured using Ellman's reagent. N-Ethyl maleimide, a well known sulfhydryl binding agent, served as a positive control and no reactive sulfhydryls were detected after NEM treatment of tubulin. There was no evidence for binding of benomyl or metabolites at the colchicine binding site. Overall the data indicated that BIC and benomyl chemically produce similar effects on the tubulin protein and that BIC may contribute to benomyl-induced inhibition of tubulin polymerization.

1150 ASSESSMENT OF THE EFFECTS OF MONOETHYLHEXYLPHTHALATE (MEHP) AND MONOBUTYL PHTHALATE (MBP) ON GENE EXPRESSION IN FETAL RAT TESTES EXPLANT CULTURES BY TRANSCRIPTION PROFILING.

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To facilitate comparison of the effects of phthalates on male reproductive organ development in vitro and in vivo we investigated the effects of phthalate metabolites monoethylhexyl phthalate (MEHP) and monobutyl phthalate (MBP) in an in vitro

fetal rat testes explant culture model. The aim of the study was to test the effects of MBP and MEHP on gene expression and testosterone (T) synthesis in order to assess a possible relationship between these end points. Gestational day (GD) 14.5 whole fetal testes explants were cultured for 72hr in the absence or presence of MEHP and MBP (1, 10 and 100 μM). Toxicity was assessed by counting gonocyte numbers. T was measured in the culture medium by radioimmunoassay. RNA was isolated, labeled and subjected to transcription profiling analysis on whole rat genome oligonucleotide microarrays. MEHP caused a dose-dependent decrease in explant T production and gonocyte numbers. By contrast MBP had little or no effect on gonocyte numbers and only a slight inhibitory effect on T. MEHP (1-10 μM)-exposed explants showed repression of gene expression in pathways involved in steroidogenesis (CYP17a, FRDX1, EBP) and cholesterol biosynthesis/transport (HMGCS, FDPS, CYP51a). This effect was less marked in MBP (1-100 μM)-exposed explants. The inhibitory effect of MEHP on CYP17a RNA expression was concentration-dependent. Fatty acid metabolism genes regulated by peroxisome proliferator receptor alpha (PPARα) were induced in fetal testes explants exposed to MEHP (10 μM) coincidently with repression of steroidogenic factor 1 (SF-1)-regulated genes. This in vitro model may provide a mechanistically-relevant tool for examining the effects of other phthalates or their metabolites on end-points relevant to testicular development.

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1151 THE EFFECT OF INDIVIDUAL VS GROUP HOUSING ON GROWTH, ONSET OF PUBERTY, AND REPRODUCTIVE ORGAN DEVELOPMENT IN MICE.

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Over the last decade numerous rodent developmental and reproductive toxicity studies have been conducted on endocrine-active substances. In 2000, the NTP and NIEHS convened an expert panel to review effects from these studies and conflicting results were reported. A recommendation from the panel included examination of housing conditions. This research studied the impact of individual and group housing (4/cage) for up to 4 months on growth, onset of puberty, and reproductive organ development in male and female CD-1 mice. Phase I examined individual-housed mice versus group-housed mice. Phase II assessed responses of different housing on development following in utero exposure to 17b-Estradiol (E2). Phase III examined group-housed mice using littermates, group-housed mice using non-littermates, individual-housed mice, and combined individual/group-housed mice. Results: (1) individual-housed male mice had larger reproductive organs and lower body weights than group-housed mice using non-littermates. (2) Individual-housed mice are comparable to group-housed mice when littermates are used. (3) No statistically significant differences were observed between individual-housed mice and group-housed mice for developmental effects following in utero exposure to E2. (4) Onset of puberty is not impacted by housing conditions for either sex. (5) Adrenal weights are larger for individual-housed mice and group-housed mice using littermates compared to group-housed mice using non-littermates. (6) Males showed greater differences between housing conditions than females. (7) There was evidence of partial reversibility for body weight and reproductive organ weights when mice were switched from group housing to individual housing. Overall, this work suggests that housing conditions should be considered prior to designing reproductive toxicity studies and may help explain differences observed between laboratories conducting work in this field of research.

1152 EFFECT OF METHOXYCHLOR METABOLITE HPTE IN RAT OVARIAN GRANULOSA CELLS.

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Ovarian granulosa cells play a central role in steroidogenesis, which is critical for female reproduction. The gonadotropin follicle stimulating hormone (FSH) primarily works through the cAMP–mediated signaling pathway and regulates granulosa cell steroidogenesis. We showed previously that 2, 2-bis-(p-hydroxyphenyl)-1, 1, 1-trichloroethane (HPTE) inhibited FSH- and dibutyryl cyclic AMP (db-cAMP)-stimulated steroid production and mRNA levels of steroidogenic pathway enzymes in rat granulosa cells. However, differential inhibition by HPTE in FSH- and db-cAMP-stimulated granulosa cells was observed. Therefore, the objective of this study was to analyze global gene expression profiles in basal, FSH-, or db-cAMP-stimulated granulosa cells, following exposure to HPTE. Immature (23-27d old) rat granulosa cells were exposed to 0, 1,5 or10 μM HPTE in the presence or absence of 3 ng/ml FSH or 1 mM db-cAMP for 48 h. Total RNA was isolated for microarray analysis (n = 3). Data was analyzed using ArrayAssist microarray suite and Database for Annotation Visualization and Integrated Discovery 2007. Investigation of the steroidogenic pathway genes showed that HPTE up regulated steroidogenic acute regulatory protein and hydroxysteroid 11-beta dehydrogenase. We observed a trend that HPTE inhibits several steroidogenic pathway genes (e.g., CYP19A, CYP11A and luteinizing hormone receptor) and that HPTE is more effective in inhibiting the mRNA levels of steroidogenic enzymes in FSH-stimulated cells versus db-cAMP stimulated cells, confirming previous observation. Up regulation of genes was observed in the extracellular matrix receptor, cell communication, and focal adhesion pathways. The cell cycle pathways exhibited 9 genes that were down regulated. Further data analysis is underway to identify differentially regulated pathways by HPTE between FSH-and cAMP-stimulated treatment groups.Supported by ES005022&NIEHS grant ES07148-18

1153 CELL CYCLE REGULATORS IN MOUSE OVARIAN ANTRAL FOLLICLES ARE ALTERED BY METHOXYCHLOR EXPOSURE.

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Methoxychlor (MXC) is an organochlorine pesticide used against insects that attack fruits, vegetables, and home gardens. Human and wildlife exposure to MXC is of concern because MXC has been shown to reduce fertility in female rodents by causing ovarian atrophy and persistent estrous cyclicity. Further, MXC decreases antral follicle numbers and increases the percentage of atretic antral follicles. MXC also inhibits antral follicle growth *in vitro*. The mechanism by which MXC inhibits growth of antral follicles is unknown. Granulosa cell proliferation is required for normal follicle growth and is controlled, in part, by cell cycle regulators. This study tested the hypothesis that MXC inhibits follicle growth *in vitro* by reducing the levels of selected cell cycle regulators. Antral follicles were isolated from adult CD-1 mouse ovaries and cultured in supplemented media. Follicles were exposed to dimethylsulfoxide (vehicle) or MXC (1-100μg/mL) for 72 and 96 hr. After culture, real-time polymerase chain reaction was performed on the follicles to measure mRNA levels of cyclin D2 (*CcnD2*) and cyclin dependant kinase 4 (*Cdk4*). MXC (100μg/mL) significantly decreased *Cdk4* mRNA levels compared to control at 72 hr (control=1.34±0.14 genomic equivalents (ge); MXC=0.56±0.07 ge; n=3; p≤0.01) and at 96 hr (control=1.52±0.13 ge; MXC=0.82±0.18 ge; n=3; p≤0.02). Further, MXC (1-100μg/mL) significantly reduced *CcnD2* mRNA levels compared to control at 72 hr (control=1.06±0.03 ge; 1μg/mL MXC=0.56±0.20 ge; 10μg/mL MXC=0.23±0.02 ge; 100μg/mL MXC=0.16±0.03 ge; n=3; p≤0.05) and at 96 hr (control=1.00±0.10 ge; 1μg/mL MXC=0.41±0.09 ge; 10μg/mL MXC=0.27±0.03 ge; 100μg/mL MXC=0.27±0.04 ge; n=3; p≤0.05). Collectively these data indicate that MXC exposure *in vitro* reduces the expression of *CcnD2* in a dose-dependant manner and reduces the expression of *Cdk4* at the highest dose in antral follicles. Therefore, we postulate that MXC may inhibit the growth of antral follicles by decreasing the levels of cell cycle regulators in antral follicles, particularly *CcnD2*. Support: NIHR21ES13061, R01ES012893, and NIHHD46861

1154 DOSE DEPENDENT EFFECTS OF SERTOLI CELL TOXICANTS 2, 5-HEXANEDIONE, CARBENDAZIM, AND MONO-(2-ETHYLHEXYL) PHTHALATE IN ADULT RAT TESTIS.

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Sertoli cells are the primary cellular target for a number of pharmaceutical and environmental testicular toxicants, including 2,5-hexanedione, carbendazim, and mono-(2-ethylhexyl) phthalate. Exposure to these individual compounds can result in impaired Sertoli cell function and subsequent germ cell loss. The loss of testicular function is marked by histopathological changes in seminiferous tubule diameter, seminiferous epithelial sloughing, vacuolization, spermatid head retention, germ cell apoptosis, and altered microtubule assembly. The present study investigates dose-response relationships for these classic Sertoli cell toxicants using histopathology endpoints. Understanding the relationship between the Sertoli cell toxicant dose and its histopathologic manifestations will help establish the sensitivity of these endpoints as markers of testicular injury. The results indicate that no single histopathology endpoint was sensitive on its own in identifying altered testicular morphology resulting from toxicant exposure. However, when multiple endpoints were combined dose-response relationships could be associated with incremental alterations in histopathology. The data generated from these experiments will be useful in further investigating the effects of Sertoli cell toxicant exposure in animal toxicity studies. In addition, this work is fundamental to a planned investigation of the histopathologic and gene expression changes associated with testicular toxicant co-exposures, which may occur both occupationally and environmentally.

1155 REPRODUCTIVE AND DEVELOPMENTAL EFFECTS OF GESTATIONAL EXPOSURE TO PERFLUOROOCTANOIC ACID IN MALE CD-1 MICE.

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Perfluorooctanoic acid (PFOA) is a ubiquitous perfluoroalkyl acid, known for its grease and water-repellant properties. Developmental toxicity has been described in mice gestationally exposed to PFOA. This study examines the long-term effects of prenatal PFOA exposure in male mice. Pregnant CD-1 mice were gavaged with water, 0.01, 0.1, 0.3, 1, or 5 mg PFOA/ kg body weight (BW) on gestation days 1-17. On postnatal day (PND) 1, litters were culled to 10 pups, with 4-5 males per dam. Male pup BW on PND4 was significantly lower than controls in a dose dependent manner ($p<0.05$). These effects persisted through weaning. Preputial separation (PPS, a marker for puberty) occurred earlier ($p<0.05$) in the 0.3 mg/kg treatment group (28.1+/-0.1d), but was delayed ($p<0.05$) in the 5 mg/kg treatment group (30.0+/-0.3d) compared to controls (28.7+/-0.2d). BW at PPS was lower ($p<0.05$) in the 5 mg/kg treatment group (20.7+/-0.7g) compared to controls (24.8+/-0.4g). There were no significant differences in BW at one year or at necropsy (18 months). At necropsy, serum was collected for hormone measures, and spleen, liver, seminal vesicle, prostate, and testes were weighed and collected for histopathological analysis. There were no differences in mean tissue mass between treated and control males. However, there was a difference in incidence of enlarged seminal vesicle (mass > control mean + two SE) across treatment groups (Kruskal-Wallace Test, $p<0.01$), as well as a significant association between the incidence of enlarged seminal vesicles and treatment (Fisher's Exact Test, $p<0.05$). The association of enlarged seminal vesicle with treatment suggests gestational PFOA exposure may affect male accessory gland processes later in life. (This abstract does not necessarily reflect EPA policy).

1156 ADULT OUTCOMES OF GESTATIONAL OR ADULT EXPOSURE TO PERFLUOROOCTANIC ACID (PFOA) IN FEMALE CD-1 MICE.

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PFOA, an environmentally persistent chemical detected in humans and wildlife, is a surfactant with wide consumer and industrial applications. Developmental exposure to PFOA is associated with decreased body weight in neonates as well as other later life effects. This study addresses whether prenatal exposure to PFOA also leads to adult weight gain and changes in organ weights. Timed pregnant CD-1 mice (n>30 per dose group) were exposed to PFOA (0.01, 0.1, 0.3, and 1 or 5 mg/kg/day) via oral gavage over days 1 to 17 of pregnancy. At post-natal day 1 (PND1), litter weights were recorded and the 5 mg/kg litters weighed significantly less than control animals. At 18 months, the animals were sacrificed and body weight, organ weight, and fat weights recorded. There was a significant increase in both body weight (0.1, 0.3, and 1 mg/kg) and inter/subscapular brown fat in the 1 mg/kg PFOA group when compared to control animals. Liver, spleen, abdominal white fat and liver to body weight ratio were not significantly different in any of the treatment groups. A subset of the gestationally exposed females ovariectomized (ovx) at PND21 were followed concomitantly with the intact animals. The body weights of these ovx animals showed no significant difference versus intact animals when compared within dose groups. A final group of age-matched females were dosed as adults (0, 1, 5 mg/kg PFOA) for 17 days and followed out to 18 months. These adult exposed animals showed no significant increase in body weight and no significant changes in organ or fat weight at 18 months when compared to controls. These data show a critical window of exposure to PFOA leads to increased weight gain as an adult. Whether increased body weight associated with prenatal PFOA exposure is associated with other health effects later in life is under investigation (This abstract does not necessarily reflect EPA policy.).

1157 EVALUATION OF TWO-GENERATION REPRODUCTIVE TOXICITY OF FLAME RETARDANT HEXABROMOCYCLODODECANE (HBCD) IN RATS.

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Male and female rats were fed a diet containing HBCD at 0, 150, 1500 or 15000 ppm throughout the study beginning at the onset of a 10-week pre-mating period and continuing through the mating, gestation, and lactation periods for two generations. At 15000 ppm, decreased body weight and body weight gain associated with reduced food consumption were found in F1 males and females. Absolute and relative weights of the liver in F0 male adults and F1 and F2 male and female weanlings at 1500 ppm and higher, and in F1 male and F0 and F1 female adults at 15000 ppm, and of the thyroid in male and female F0 and F1 adults at 15000 ppm were increased. The incidence of rats with decreased thyroid follicles size was increased in F0 and F1 males and females at 1500 ppm and higher. An increase in serum TSH levels in F0 and F1 females at 1500 ppm and higher, and a decrease in serum T4 levels in F0 males and females at 15000 ppm were noted. The number of the primordial follicles in the ovary of F1 females was reduced at 1500 and 15000 ppm. No changes were observed in the estrous cyclicity, copulation index, fertility index, gestation index, delivery index, number of implantations, precoital interval or gestation length in any generations and in the sexual development in the F1 generation at any doses of HBCD. In F2 pups, low incidences of the completion of eye opening in males at 15000 ppm and in females at 1500 ppm and higher, and of completed mid-air righting reflex in females at 15000 ppm were found. There were no compound-related changes in the number of pups delivered, sex ratio of pups, anogenital distance, surface righting reflex, negative geotaxis reflex, pinna unfolding or incisor eruption in F1 and F2 generations. The viability index of F2 pups and the body weight of male F1 and F2 pups and female F2 pups were decreased at 15000 ppm. The data indicate that the NOAEL of HBCD in this study was 150 ppm (10.2 mg/kg bw/day)

1158 EFFECTS OF *IN UTERO* AND LACTATIONAL EXPOSURE TO 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) ON REPRODUCTIVE FUNCTION IN MALE RHESUS MONKEYS.

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We examined the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on reproductive function of the male offspring of rhesus monkeys. Pregnant animals received an initial dose of TCDD [0 (vehicle control), 30, or 300 ng/kg subcutaneously] on Day 20 of gestation and then monthly maintenance doses (5% of the initial dose) until 90 days after delivery. At the age of sexual maturity (6 or 7 years old), body weight changes, morphological development of the reproductive organs, serum hormone concentrations (testosterone and inhibin B), and semen indices were evaluated in 4 males in the control, and 6 and 5 males in the 30 and 300 ng/kg groups, respectively. In the 300 ng/kg group, body weight gain from the age of 3 years was suppressed by approximately 10% compared with the control. Testicular diameter and penile length were not significantly different among the 3 groups; however, a tendency toward a dose-proportional decrease in testosterone and a dose-proportional increase in inhibin B were noted in the 30 and 300 ng/kg groups. Although individual values varied widely in semen analysis, sperm activity and sperm viability showed a tendency toward a dose-proportional decrease. Furthermore, sperm count in the 300 ng/kg group was decreased by approximately one-third that of the control. Accordingly, it was considered that exposure to TCDD during the gestation and lactation periods could affect reproductive function in male rhesus offspring.

1159 ATSDR'S ACUTE MINIMAL RISK LEVEL FOR HEPTACHLOR.

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The Agency for Toxic Substances and Disease Registry has recently completed its re-evaluation of the toxicity of heptachlor and its metabolite heptachlor epoxide. Heptachlor is an organochlorine cyclodiene insecticide that was extensively used prior to 1970 to kill termites, ants, and soil insects in seed grains and on crops. Currently, the only permitted use of heptachlor is for fire ant control in power transformers. Although the use of heptachlor is restricted, the general population can be exposed to heptachlor or heptachlor epoxide through the ingestion of contaminated food. A number of studies have examined the toxicity of heptachlor following acute-duration oral exposure; many of the toxicity studies are limited by the lack of statistical analysis and poor reporting of the observed effects, including incidence data. Despite the limitations in the studies, the acute database does identify several targets of toxicity including the liver, nervous system, reproductive system, and the developing offspring. The reproductive system appears to be the most sen-

sitive target following acute oral exposure. Reduced fertility and an increase in resorptions were observed in female rats administered 1.8 mg/kg/day of heptachlor in groundnut oil for 14 days and mated to unexposed males. Decreased body weight and righting reflex were observed in pups with gestational exposure to 4.2 or 4.5 mg/kg/day. Minimal liver effects and neurological effects were observed in rats administered 7 mg/kg/day for 14 days. An acute-duration oral minimal risk level (MRL) was derived based on the LOAEL of 1.8 mg/kg/day for reproductive effects. This LOAEL was divided by an uncertainty factor of 1000 (10 for use of a LOAEL, 10 for extrapolation from rats to humans, and 10 for human variability) and a modifying factor of 3 (to account for the use of a serious end point) resulting in an MRL of 0.0006 mg/kg/day.

1160 ABSENCE OF DEVELOPMENTAL TOXICITY IN EMBRYOFETAL AND PRE- AND POSTNATAL DEVELOPMENT STUDIES IN CYNOMOLGUS MACAQUES FOLLOWING ANTAGONISM OF IL-12/23 ACTIVITY WITH USTEKINUMAB (CNTO 1275).

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Ustekinumab is a human antibody that neutralizes the bioactivity of human and non-human primate IL-12 and IL-23. It is currently being studied for the treatment of psoriasis. To identify potential adverse effects of ustekinumab on the conceptus, IV and SC embryofetal developmental (EFD) toxicity studies and a combined SC EFD/pre- and postnatal development (EFD/PPD) study were conducted in cynomolgus macaques. In EFD studies pregnant macaques were administered ustekinumab during organogenesis at doses up to 45 mg/kg weekly (IV) or twice weekly (SC). In the EFD/PPD study, pregnant macaques were administered SC injections of ustekinumab twice weekly at 22.5 and 45 mg/kg from the beginning of organogenesis, through delivery and for the first 33 days of the lactation period. Postnatal development of the F1 macaques was evaluated for 6 months. No maternal toxicity, difficulties at delivery or adverse effects on duration of pregnancy were observed. No ustekinumab-related teratogenicity or developmental delays was observed in fetuses or F1 macaques. No adverse effects of ustekinumab on the development of the immune system were observed in fetuses or F1 macaques based on: immunophenotyping of circulating lymphocytes; evaluation of T-cell and B-cells distribution in lymphoid tissues; or characterization of neoantigen responses and delayed type hypersensitivity responses of juvenile F1 macaques. Toxicokinetic analyses confirmed maternal and fetal exposure to ustekinumab at the time of C-section in EFD studies indicating that ustekinumab crossed the placenta. Following delivery, ustekinumab was present in maternal milk and in serum of all dams and F1 macaques at high concentrations. Ustekinumab exposure persisted through the 6-month postnatal period. In summary, ustekinumab was well tolerated by pregnant macaques and their offspring at dose levels 45-fold higher than an intended clinical dose.

1161 SUBLETHAL TCDD EXPOSURE DURING EARLY STAGES OF DEVELOPMENT INDUCES CRANIOFACIAL, CARDIAC, AND REPRODUCTIVE TOXICITY IN ADULT ZEBRAFISH.

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Recent evidence suggests that exposure to sublethal levels of toxicants during early life can influence developmental programming, resulting in permanent functional changes not realized until adulthood. TCDD is known to adversely affect early development and reproduction in nearly all vertebrates; however, little is known about the long-term effects following sublethal exposure. Zebrafish were exposed to various concentrations of TCDD during early life stages (waterborne 25-200 ppt, 0-7 weeks) to determine if TCDD induces latent toxicity in adults. Only those in the low-dose TCDD groups (25 and 50 ppt) survived past juvenile stages. While exposure to these sublethal concentrations of TCDD did not alter growth or induce overt signs of toxicity in adults, they had shorter jaws and some had minor fin necrosis. Histopathology revealed alterations at the light microscopic level in the liver, heart, ovary and boney structures of the head. Exposure to 25 ppt TCDD impaired reproductive success. Egg production was reduced by 60% and fertilization success by 8%. TCDD-treated males contributed more than females to impaired reproductive capacity, while both TCDD-treated males and females contributed to poor offspring survival. Offspring from 25 ppt TCDD-treated fish showed increased mortality. Surviving larvae showed a 5% reduction in growth, 27% increase in blue sac syndrome, and 80% failed to inflate the swim bladder. Preliminary results suggest reproductive success of the F1 generation is also reduced. These findings support the hypotheses that exposure to sublethal levels of TCDD during early life stages can adversely affect health and reproductive capability of adults and their offspring. These results will enable us to develop models for correlating exposure to TCDD during early life with adult-onset of disease and reproductive dysfunction.

1162 THE PHTHALATE ESTERS: PHARMACODYNAMIC (PD) *VS.* PHARMACOKINETIC (PK) FACTORS AFFECTING POTENCY IN TESTOSTERONE-MEDIATED DEVELOPMENTAL EFFECTS.

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Human exposure to phthalates occurs as a mixture of diesters with varying activity towards testosterone-dependent development. Dibutyl (DBP), diethylhexyl (DEHP), and butylbenzyl (BBP) phthalate disrupt sexual development in the male fetal rat. Dimethyl (DMP), diethyl (DEP) and dioctyl (DOP) phthalate do not show the same testicular effects as DEHP and DBP. It is not known if this varied potency in vivo results from PK or PD differences. To examine this, we compared active metabolite disposition, in vivo toxicity and in vitro dose-response data. Fetal testes from rats given DMP, DEP, DBP, DEHP, and BBP (500 mg/kg-d, GD12-19) were analyzed for the corresponding monoester (MMP, MEP, MBP, MEHP, MBeP). Testes MMP and MEP levels were 2-40 fold higher than MBP and MEHP. BBP exposure led to very low concentrations of MBeP, but similar levels of MBP to those seen after DBP exposure. In vitro progesterone (PG) assays were performed with MA-10 cells to determine direct effects on Leydig cells. MBP and MEHP showed similar PG inhibition (IC50's: 9 and 6 uM). MMP and MEP did not inhibit PG at concentrations up to 100 uM. Thus, MMP and MEP are present in the fetal testes after maternal exposure, but do not have the same inhibitory activity as longer chain phthalates. MOP showed similar PG inhibition to MBP, suggesting that a lack of effect in vivo may be due to kinetics. MBeP did not inhibit PG in vitro suggesting that developmental changes seen with BBP exposure result from preferential hydrolysis of BBP to MBP in the rat. BBP may be of less concern for human exposure, as hydrolysis in man favors MBeP. Together, these studies suggest that risk assessment efforts for combined phthalate exposure should include DBP, DEHP, and DOP. DMP, DBP, and BBP can be excluded from testosterone-dependent endpoints, as they do not exhibit this mode of action.

1163 SODIUM PERFLUOROHEXANOATE: ORAL REPEATED DOSE SUBCHRONIC, ONE-GENERATION REPRODUCTION, GENOTOXICITY, AND DEVELOPMENTAL TOXICOLOGY.

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Sodium perfluorohexanoate (PFHxNa) was evaluated in subchronic, one-generation reproduction, genotoxicity, and developmental toxicity studies. In the subchronic/one-generation reproduction study, four groups of young adult male and female Crl:CD(SD) rats were administered PFHxNa daily by gavage at dosages of 0, 20, 100, or 500 mg/kg/day. Rats were dosed for 90 days and evaluated after one- and three- month recovery periods. In the developmental study, time-mated female rats were dosed via gavage on GD 6-20 with the same doses of PFHxNa used in the subcrhonic study. On GD-21 rats were euthanized and fetuses were examined for soft tissue and skeletal alterations. The NOAEL for subchronic toxicity was 20 mg/kg/day, based on nasal lesions observed at 100 and 500 mg/kg/day. The relevance to humans is unknown. The NOAEL for reproductive toxicity was 500 mg/kg/day, the highest dose tested. No test substance-related effects were observed on reproductive parameters. The NOEL for P1 adult rats was 20 mg/kg/day (reduced body weight/gains in males at 100 mg/kg/day). The NOEL for F1 offspring was 100 mg/kg/day (reduced pup weights during lactation at 500 mg/kg/day). The NOEL for F1 adults was 100 mg/kg/day, (reduced body weight/gains in F1 males and females and reduced food consumption in F1 adult males at 500 mg/kg/day). Genotoxicity studies of PFHxNa indicated no mutations in the bacterial reverse mutation assay or chromosome aberrations in human lymphocytes. In the developmental study, there were no test substance-related deaths or gross findings in the dams at any dose. The NOEL for the developmental study was 100 mg/kg/day (maternal toxicity observed as decreased body weight and food consumption and developmental toxicity based upon decreased fetal weights was observed in rats administered 500 mg/kg/day). PFHxNa is therefore concluded not to present a reproductive or developmental hazard. The lowest NOEL representing all of the studies described above was 20 mg/kg/day.

1164 TOXICOLOGY AND SENESCENCE: BASELINE VARIABILITY AND TOLUENE EFFECTS ON MOTOR FUNCTION IN AGING BROWN NORWAY RATS.

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The rapidly expanding population of older adults raises concern in EPA over aging-related vulnerability to environmental exposures. Deficits in motor function are frequent with advancing age. An increase in interindividual variability is also commonly accepted. Increased variability due to aging could complicate vulnerability assessment. Research was therefore undertaken to quantify senescence-related changes in motor activity and the effect of toluene. Male Brown Norway rats (from NIA) were tested as young (4-5 mo), middle-age (12-13 mo) or old (24-25 mo) adults. Motor activity was recorded in a photocell chamber during 30-min sessions. Each rat received corn oil (4 ml/kg) and toluene (1000 mg/kg) p.o., 30 min before testing. Toluene effects were expressed relative to each rat's vehicle-control data. Combining three experiments yielded group sizes of 18-21 animals. Under baseline conditions, motor activity (horizontal and vertical) decreased with age. Interindividual variability was greatest in the old rats, and least in middle-age rats. Toluene increased horizontal activity by approximately 45% in young and middle-age rats, and by about 150% in the old rats. Variability in the effect of toluene was greatest in the old rats, and equivalent in the two younger groups. Toluene effects on vertical activity (decreases) were irregular, with no clear pattern related to age. Further comparison of toluene effects on horizontal activity, using z-scores based on young rats as the reference group, resulted in means and SDs of 0.0 and 1.0 (young), 0.1 and 1.0 (middle age), and 1.7 and 3.0 (old). With a threshold for toluene's adverse effect (hyperactivity) set at z=1.65, the increased risk of hyperactivity was estimated to be 22% for the middle-age rats and in excess of 900% for the oldest rats. These results provide quantitative support for both the increased variability and enhanced vulnerability of senescent organisms to toxic substances.
This is an abstract of a proposed presentation; the information does not necessarily reflect Agency policy.

1165 VALIDATION OF A MORBIDITY SCORE IN A STUDY OF BOTULINUM TOXIN A.

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Guidelines for laboratory animal care dictate the use of data-based criteria that are predictive of impending death to implement timely euthanasia cases where the pain and distress category is E. The morbidity evaluation of these animals involves multivariate data including observational, biological and behavioral variables measured repeatedly. In this research we develop a composite score using methodology based on desirability functions similar to Coffey et. al (Regulatory Toxicology and Pharmacology, 2007) with validation of the composite score based on its statistical relationship to instantaneous hazard of death. Such endpoints were examined on CD-1 mice exposed to Botulinum toxin A in 5 dose groups with 10 mice per group which were monitored twice daily for up to 5 days. Body temperature measurements and ordinal scores for piloerection (present/not present), muscle tone (normal, moderate loss, severe loss), respiration (normal, thoracic tachypnea, abdominal tachypnea, dyspnea), and activity (normal, decreased, little or no activity) were synthesized into a single unitless composite score with ranges between 0 (most severe) and 1 (normal levels of response). The final morbidity score for animals that died (56%) had a mean of 0.087 (SD=0.079; median=0.068; min= 0.005; max= 0.261). For animals that recovered (44%) the mean score was 0.517 (SD=0.114; median=0.504; min= 0.325; max= 0.821). The model for log relative hazards was curvilinear (approximated with a quadratic model). The hazard of death was significantly related to the morbidity score ($p<0.001$) thus validating the score. Data from a second similarly-designed study were used to evaluate the reproducibility of the result. The shape of the relationship between the morbidity score and the hazard of instantaneous death across the two studies was not significantly different ($p=0.88$), indicating a reproducible relationship.

1166 DNA ADDUCT FORMATION OF LOW DOSE 13C2H2 LABELED METHYLMETHANE SULPHONATE.

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It is well established that mutations in genes related to cell proliferation and death are critical events in the development of cancer. Background mutations are an inherent part of any mutation assay and are thought to be the result of endogenous DNA damage. When extrapolating risks of cancer for genotoxic agents, determining where the biology of mutagenesis is driven by exogenous exposure represents critical information for risk assessment. Methylmethane sulphonate (MMS), an experimental alkylating agent, directly interacts with DNA donating a methyl group to the N 7 position of Guanine. The goal of this study was to quantify N7-Me-Gua adducts that correspond to concentrations of MMS that have been reported to generate a non-linear mutagenic dose response curve. N7 levels directly attributed to MMS methylation were measured by exposing AHH-1 cells to concentrations of [13C2H2]labeled MMS ranging from 0.5 μg/mL to 2.0 μg/mL for 24 hours. Following DNA isolation and neutral thermal hydrolysis, N7 MeGua and [13C2H2] N7-Me-Gua adducts were analyzed using LC-MS/MS. Endogenous N7-MeGua was at the limit of quantitation. [13C2H2] N7-Me-Gua adducts increased linearly with dose ranging, from a mean 167 ± 24 adducts/10^8nt in cells exposed to 0.5 μg/mL MMS, to 571 ± 144 adducts/10^8nt for cells exposed to 2.0 μg/mL MMS. Data from this study demonstrates that the formation of exogenous adducts is linear from low to high dose. Considering that mutations are initially at background levels and do not significantly increase until exposures of 1.25 μg/mL or higher, this suggests, that mutagenesis is driven by endogenous processes at exposures of 1μg/mL MMS or lower.
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1167 *IN VITRO* ASSESSMENT OF HUMAN SKIN METABOLISM OF AROMATIC AMINE HAIR DYES.

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In vitro skin metabolism is a key consideration for the risk assessment of hair dyes and potentially all other chemicals intended to be used in dermal applied consumer products. In the case of aromatic amine hair dyes, elucidation of the metabolic pathway(s) is of particular interest, since these molecules contain reactive amino groups. We investigated the skin metabolism of oxidative hair dye precursors in vitro using the human keratinocyte cell line HaCaT. We could experimentally demonstrate that HaCaT cells express enzymes, such as N-acetyltransferase 1 playing a key role for the metabolism of this amino group(s). Moreover, our findings were confirmed by data from rat ADME studies following dermal application as well as by results obtained with human skin ex vivo. Based on this background information, we developed a generic protocol for the investigation of human skin metabolism in vitro using HaCaT cells. Main aspects of the protocol include a simple and rapid work-up procedure and a set of analytical HPLC methods which allow for the analysis of typical metabolite(s) and of the parent compound in a single run. Applying this new experimental approach, we could show that HaCaT cells represent a promising model to mimic key characteristics of the metabolizing capacity of human skin. Specifically, for common hair dye precursors with p-phenylenediamine and aminophenol structure we found that N-acetylation represents the predominant metabolic pathway in the skin. We conclude that this approach allows to perform a cost- and time-effective screening of the dermal metabolite profile.

1168 HUMAN AND RAT PRIMARY HEPATOCYTE CULTURES FOR THE STUDY OF PEROXISOME PROLIFERATOR-ACTIVED RECEPTOR α (PPARα) AGONISTS ACTIVITY IN ENVIRONMENTAL CHEMICALS.

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Uncertainties exist in the toxicological assessment of potential PPARα agonists. For example it is not clear if human liver is refractile to some or to all PPARα agonists pertaining to peroxisome proliferation; nor is it known if PPARα agonists induce oxidative stress and changes in tryptophan-NAD pathway in human. We designed human and rat primary hepatocyte cultures to address these uncertainties. Clofibrate (CLO), di(2-ethylhexyl) phthalate (DEHP), perfluorooctanoic acid (PFOA) and perfluoroctane sulfonate (PFOS) were used as test chemicals and PCB 153 as negative control. Hepatocytes obtained from rats by collagenase perfusion were added to MATRIGEL-coated 24-well plates at 350,000 cells/well. Human hepatocytes in collagen-coated 24-well plates (~ 80% confluent) were purchased from XenoTech and BD Biosciences. Hepatocytes were incubated at 37 C for 24 h in a humidified atmosphere of 5% CO2/air. Fresh media spiked with chemicals in DMSO or DMSO alone were then added. Media were changed daily. After 4 days of incubation, cell viability was assessed microscopically and by LDH leakage, and peroxisome response was assessed by measuring the biomarker palmitoyl CoA Oxidase (PCO). With rat hepatocytes, PFOA at 0.2 mM produced 800% increase in PCO over DMSO control, followed by DEHP at 350% and CLO at 270%. No increase was observed with PCB 153 at 1 mM. Human hepatocytes were much less responsive, with only CLO eliciting 25-38% increases in PCO. Hepatocyte viability was unaffected by these chemicals. PFOS effect was difficult to assess as hepatocyte viability was significantly compromised at 0.1 - 0.5 mM. This indicated that acute cytotoxicity of PFOS is in itself a toxicological concern. In summary, we have

designed rat and human hepatocyte cultures that are viable and responsive to PPARα agonists in a species-related manner. This dual species system will be used to (1) study effects on oxidative stress and the tryptophan-NAD pathway, and (2) to evaluate environmental chemicals for potential PPARα agonist activity.

1169 AN *IN VIVO* PHOTOCARCINOGENESIS STUDY DEMONSTRATING THE VALIDITY OF THE SCUP-M ACTION SPECTRUM FOR UVR-INDUCED SKIN CANCER.

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The recently published Commission Internationale De L'eclairage (CIE) Standard Action Spectrum for Non-Melanoma Skin Cancer (S 019/E:2006) is based on studies in laboratory animals and the resulting Skin Cancer Utrecht Philadelphia-man (SCUP-m) action spectrum (weighting function) modified to account for anatomical differences in human skin. Recent spectral and mathematical analyses confirmed that the SCUP-m weighting function accurately describes the tumor responses attributable to the ultraviolet radiation (UVR) sources used, and predicted that blocking the UVR scatter from the xenon long-arc solar simulator (i.e., including radiation < 290 nm) would have little or no measurable impact on tumor response in hairless mice. We now provide data from the first direct laboratory test of that hypothesis. The methodology differed from previous studies only by virtue of modifying the lamp housing to prevent the small but measurable amount of scattered radiation from reaching the mouse cages. Mice in two groups received weekly (Monday-Friday) UVR exposures totaling either 600 or 1200 Robertson Berger Units, equivalent to 300 or 600 J/m^2 (CIE erythema-weighted), respectively. The median latent periods (for tumors > 1 mm planar diameter) were 37 and 23 weeks respectively. This response is well within the parameters of our published historical control data, all of which were based on the xenon lamp prior to its modification. This direct evidence lends further experimental support to the spectral specifications in CIE Standard S 019/E :2006. The result further discounts recent speculation that UVR scatter from the xenon UVR source substantially contributed to the observed tumor response, and thus skewed the calculated weighting function.

1170 ORAL BIOAVAILABILITY OF CYCLOTRIMETHYLENETRINITRAMINE (RDX) FROM CONTAMINATED SITE SOILS IN RATS.

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Cyclotrimethylenetrinitramine (RDX), a commonly used military explosive, is present as contaminant of soil and water at Army facilities and ranges. This study was conducted to determine the relative oral bioavailability of RDX in contaminated soil and to develop a method to derive bioavailability adjustments for risk assessment. Adult male Sprague-Dawley rats (Charles River Laboratories) pre-implanted with femoral artery catheters were dosed orally with gelatin capsules containing either pure RDX or an equivalent amount of RDX in contaminated soils from Louisiana Army Ammunition Plant (LAAP) (2300 μg/g of soil) or Fort Meade (FM) (670 μg/g of soil) . After dosing, blood samples were collected from the catheters at 2, 4, 6, 8, 12, 24 and 48 hrs. RDX levels in the blood were determined by gas chromatography and the peak concentration occurred at 6 hrs after dosing for the animals receiving the 1.24 mg/kg neat or soil-bound (LAAP soil) dose. Rats dosed with FM soil contaminated showed peak levels of RDX in blood at 6 hrs while the equivalent levels of neat RDX (0.2 mg/kg) showed peak absorption at 4 hrs. The relative bioavailability of RDX from LAAP soil was about 25 % less than the equivalent neat RDX. The bioavailability of RDX from FM soils showed a 10-15% decrease for the earlier sample times only. Thus, the oral bioavailability of RDX in soil was reduced when compared with the neat compound but the reduction in bioavailability varied with soil type.

1171 *IN VIVO* BIOAVAILABILITY OF ARSENIC IN COAL COMBUSTION BY-PRODUCTS.

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Coal combustion by-products (CCBs) are unburned residues that are left when coal is burned to produce power. These residues are considered to be by-products because there are many beneficial re-uses for these materials such as concrete, structural fill, and waste stabilization. A survey of metals concentrations in CCB-containing fill used as roadbed in a residential area has indicated that arsenic (As) concentrations can range from 4.7 to 97 mg/kg. Assessment of potential health risks associated with exposure to CCBs in these settings can benefit from knowledge of the amount of As absorbed from the gastrointestinal (GI) tract into the body. A study using juvenile swine as test animals was performed to measure the GI absorption of arsenic from two samples of CCB-containing fill: Test Material 1 contained 217 mg/kg As in the fraction passing through a 250 um sieve and Test Material 2 contained 80 mg/kg As in the 250 um seived fraction. The relative bioavailability of As was assessed by comparing the absorption of As from the test materials to that of a reference material (sodium arsenate). Groups of five swine were given oral doses of sodium arsenate or test material twice a day for 14 days; a group of five non-treated swine served as a control. The amount of arsenic absorbed by each animal was evaluated by measuring the amount of arsenic excreted in the urine (collected over 48-hour periods beginning on days 6, 9, and 12). The urinary excretion fraction (UEF) (the ratio of the amount excreted per 48 hours divided by the dose given per 48 hours) was calculated for both the test soil and sodium arsenate using linear regression analysis. The relative bioavailability (RBA) of arsenic in the test soil compared to that in sodium arsenate was calculated as a ratio (UEFtest/UEFref). The As RBA estimates are approximately 72% for Test material 1 and 50% for Test Material 2. These results indicate that the As in the test materials are not as well absorbed as soluble arsenic, and these RBAs are below the default regulatory assumption of 100% absorption.

1172 REDUCTION OF STRAWBERRY PESTICIDE RESIDUES USING CALYX REMOVAL AND A WATER RINSE.

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Pests and people both consume strawberries. Pesticide residues on plants are regulated using preharvest intervals to very effectively protect harvesters from both short and long-term no observed adverse effect levels (NOAELs) of pesticide exposure. Still lower levels of pesticide exposure are associated with eating produce. Residues below tolerances are far below levels that could cause toxicity in rats or humans even with exaggerated (or impossible) rates of consumption. Experiments were performed to determine residue reduction with usual preparation of fresh strawberries. Following normal pesticide use on commercial farms in Santa Maria and Salinas, CA, one pound boxes of berries were randomly separated into 4 groups: 1) fresh, whole berries, 2) rinsed whole berries, 3) unrinsed berries minus calyx/crown, and 4) rinsed berries minus calyx/crown. Rinsed berries were shaken 30 sec in a closed container in 500 ml Nestle PureLife® water. Pesticides (2 or 3 a. i.) on 3 separate lots included captan, bifenthrin, carbaryl, danitol, naled (as dichlorvos), and thiamethoxam. Residues were analyzed at primuslabs.com (Santa Maria CA) using FDA Pesticide Analytical Manual (1994) residue methods (PAM 242.1). Calyx/crown removal and water rinse reduced residues to lower levels in all cases. Residues were further reduced by removal of the green, leaf-like calyx and crown of the berries and a 30 second water rinse. In decreasing order of residue reduction (based on calyx/crown: fresh): captan > bifenthrin > danitol ≈ thiamethoxam. The effectiveness of the water rinse for removal of residue (residue after 100 - water rinse/ fresh) was as follows: captan (90%) > danitol (28%) > bifenthrin (18%) > thiamethoxam (10%). Residue reduction with water is likely influenced by the chemical and physical nature of the residues. Routine preparation of fresh strawberries involving calyx/crown removal and water rinsing reduces trace pesticide levels, already less than established tolerances, and well below NOAELs for both harvesters and consumers.

1173 REDUCING ANIMAL USE IN REGULATORY TOXICOLOGY: A REVIEW OF CURRENT WAIVER SUBMISSIONS UNDER THE EU BIOCIDES DIRECTIVE.

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Within the EU a vast programme of chemical safety reviews is in progress, under auspices of the Biocides Directive and REACH. For almost all manufactured chemicals and chemical products, toxicity data are being required within specific deadlines. Probable consequences are that many chemicals will be removed from the market due to absence of appropriate data, or that new testing must be performed. There are profound implications for animal use, and regulators are encouraged to permit non-submission ("waiving") of studies when this can be scientifically justified. This review is intended to inform applicants of potential to reduce animal use, and on associated strategies to help reassure regulators of the sufficiency of waivers. From regulatory assessments made public under the Biocides directive (for product groups "Wood Preservatives" and "Rodenticides" to date), the extent of acceptance of waivers is reviewed. Types of study for which waivers are submitted, and some limited information on the rationale for such waivers, are described.

1174 PHASE I TO II CROSS-INDUCTION OF XENOBIOTIC METABOLIZING ENZYMES: A POTENTIAL SOURCE FOR HORMETIC RESPONSES.

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It is currently hypothesized that hormetic responses to xenobiotic exposure occur as a result of overcompensation by the homeostatic control mechanisms operating in biological systems. A well-known homeostatic circuit in the cell is the gene induction network comprising phase I and II metabolizing enzymes, which are responsible for xenobiotic bioactivation and detoxification. By formulating an ordinary differential equation-based computational model, we investigated whether hormesis can result from the operation of this gene network. The model consists of two feedback and one feedforward controls. With the phase I negative feedback control, xenobiotic X activates nuclear receptor AhR to induce cytochrome P450 enzyme (CYP), which bioactivates X into reactive intermediate X'. With the phase II negative feedback control, X' activates transcription factor Nrf2 to induce phase II enzymes such as GST and GCL, etc., which participate in reactions that metabolize X' into less reactive metabolite X". The feedforward control involves phase I to II cross-induction, in which parent chemical X can directly induce phase II enzymes through AhR. We demonstrate that, as a result of the active feedforward control, a hormetic relationship readily arises between the steady-state concentration of intracellular reactive intermediate X' and that of extracellular parent chemical X. The dose response has a temporal profile comparable with homeostasis-mediated hormesis. The magnitude of the hormetic response can be enhanced by an increase in the feedforward gain, but attenuated by increases in the phase I and/or phase II feedback loop gains. Our results suggest a plausible, common mechanism for the hormetic responses observed with many mutagenic/carcinogenic xenobiotics whose activities require bioactivation by phase I enzymes. Dose responses and risk assessments associated with these chemicals need to take into consideration the coordinated homeostatic control system constantly operating in the cell.

1175 INTEGRATION OF BENCHMARK DOSE ANALYSIS WITH GENOMIC DATA TO ASSESS THE FUNCTIONAL EFFECTS OF CHEMICAL EXPOSURE.

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Microarray technology allows a comprehensive examination of molecular changes following chemical exposure and can provide insights into key events in the mode-of-action. Despite the potential utility of the technology, the application of microarrays to dose-response assessment has been limited due to the lack of analysis methods that examine the dose-response information in a risk assessment context. To address this limitation, a method was established that combines traditional benchmark dose (BMD) calculations with gene ontology (GO) classification in the analysis of dose-response data from microarray experiments. The method involves a stepwise analysis beginning with a one-way analysis of variance to identify genes that demonstrate significant dose-response behavior. The second step fits the gene expression data to a selection of statistical models and selects the model that best describes the data with the least amount of complexity. The selected model is then used to estimate the BMD at which the expression of the gene significantly deviates from that observed in control animals. Finally, each gene is matched to its corresponding GO categories and summary values are calculated that characterize the dose-dependent behavior for each biological process and molecular function. As a case study, gene expression changes in the rat nasal epithelium following acute formaldehyde exposure were analyzed. The BMD estimates for the GO categories related to cell proliferation and DNA damage from our approach were similar to those measured in previous studies using cell labelling indices, DNA-protein cross-links and rat nasal tumors. The lowest mean BMD estimate, 1.57 ppm, was for microtubule polymerization. The combination of microarray technology with these analysis methods results in a unique risk assessment tool that provides both a comprehensive examination of potential key events in the mode-of-action for a chemical and reference doses at which these key events occur.

1176 ACUTE INHALATION TOXICITY: PHYSIOLOGICAL TIME AND SPECIES-SPECIFIC HALF-LIFE AS DETERMINANTS OF ANIMAL-TO-HUMAN EXTRAPOLATION OF TOXICOLOGICALLY EQUIVALENT COMBINATIONS OF AIR CONCENTRATION AND DURATION.

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Previous theoretical work has showed that, within a species, elimination half-life largely dictates chemical-specific patterns of trade-off between air concentration and exposure duration to produce equally toxic bouts of acute inhalation exposure. Larger animals (such as humans) have systematically higher half-lives of elimination than do experimental rodents. Here I show, in a further theoretical argument, how scaling exposure durations in terms of species-specific half-lives renders animal and human exposures comparable in terms of the kinetics of uptake and normalizes air concentration/duration trade-offs such that animal experimental results can be applied to predict human acute inhalation toxicity for different combinations of air concentration and duration. The exposure/duration trade-off patterns for equitoxic exposures determined experimentally in rodents do not apply to humans unless scaled in this way. This work was funded by the U.S. Environmental Protection Agency under contracts 4C-R302-NALX and EP-06-C-000304.

1177 BENCHMARK DOSES FOR CHEMICAL MIXTURES: EVALUATION OF A MIXTURE OF 18 PHAHS.

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Benchmark doses (BMDs), defined as doses of a substance that are expected to result in a pre-specified level of "benchmark" response (BMR), have been used for quantifying the risk associated with exposure to environmental hazards. The lower confidence limit of the BMD is used as a basis for the point of departure (POD) in risk assessments, often with additional uncertainty factors included. This risk estimate, however, does not account for potential interaction of the substance with other chemicals included in human exposures. The present work developed and tested a methodology to estimate BMDs for mixtures of chemicals at fixed mixing ratios. Comparisons were made to the BMD under the assumption of additivity. Young female Long-Evans rats were dosed via gavage with 18 different polyhalogenated aromatic hydrocarbons [2 dioxins, 4 dibenzofurans, and 12 PCBs, including dioxin-like and non-dioxin-like PCBs], or a mixture with the ratio of the 18 chemicals based on environmental concentrations, for 4 consecutive days. Serum total T4 was measured via radioimmunoassay in samples collected 24 hr after the last dose. Analyses of these data (Crofton et al, EHP, 2005) suggested no departure from additivity in the low dose region of the mixture, implying a BMD for the mixture could be based on single-chemical data in an additivity model. Our objective was to evaluate this assumption. Three candidate nonlinear additivity models were considered and ranked with the AIC criteria. To calculate the BMD, the BMR was selected to be a 10% shift in T4 (expressed relative to controls). The results of a Wald-type test revealed no statistically significant difference between the BMD under additivity compared to that from the mixture data. We conclude that use of a BMD for this mixture could be based on single chemical data in an additivity model. (Partially supported by NIEHS #T32ES007334 and does not reflect USEPA policy.)

1178 DEVELOPMENT OF WEIGHTED DISTRIBUTIONS OF REPS FOR DIOXIN-LIKE COMPOUNDS: IMPLICATIONS FOR RISK ASSESSMENT.

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In their recent reevaluation of the TEF methodology, the WHO expert panel indicated that consideration should be given to developing weighted distributions of REP values to establish TEFs for each dioxin-like compound (DLC). As such, we developed a consensus-based weighting framework where greater emphasis is placed on those REP values from studies of higher quality and those that are believed to be more relevant for assessment of human health risks. In this case study, the impact of using weighted REP distributions was evaluated by calculating fish ingestion dose estimates using three approaches: WHO TEFs, point estimate TEFs based on a series of consistent percentiles from the weighted and unweighted distributions, and using the full weighted and unweighted distributions in a Monte Carlo analysis. Preliminary results indicate that while unweighted distributions tend to yield higher dose estimates than do weighted distributions, the differences are within an order of magnitude regardless of whether a consistent percentile is chosen or the full distribution is used in a Monte Carlo analysis. Dose estimates calculated using the WHO TEFs were found to be consistent with dose estimates calculated using the 50th percentile of the weighted and unweighted distributions; however, at higher percentiles, dose estimates determined based on the unweighted and weighted distributions were 1-2 orders of magnitude higher than estimates using WHO TEFs. These results indicate that use of distributions, particularly when the upper percentiles were utilized as relative potency metrics, had a greater impact on the dose estimates than did the weighting process alone. Overall, these findings suggest that use of weighted distributions of REPs could have significant implications for assessing human health risks. (This abstract does not represent USEPA policy)

1179 VALUE OF INFORMATION ANALYSIS INTEGRATING DIVERSE INFORMATION SOURCES TO ESTIMATE VARIABILITY OF GENOTYPES AND PHENOTYPES OF CYP450 ENZYMES IN METABOLISM OF CHLORPYRIFOS.

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A number of recent studies of the organophosphate pesticide chlorpyrifos (CP) metabolism have concentrated attention on the detoxification of oxon metabolite of CP by polymorphisms of PON1 and their phenotypic variation in the quantity of the enzyme. In this study we examine the initial step in the metabolism by CYP450 enzymes of CP which involves conversion to either a more toxic CP-oxon or to a relatively non-toxic compound, TCP. Four different CYP450 enzymes account for the majority on the metabolism, 1A2, 2B6, 3A4, and 2C19, and each has different rates of production of CP-oxon and TCP. The rate at which these reactions occur has been the focus of a great deal of recent research, since these rates control the amount of the CP-oxon produced and how quickly it is detoxified. Polymorphisms in the CYP450 enzymes involved in the metabolism can greatly alter these rates. We look at the genetic and phenotype variability in the CYP450s using recently published data from the scientific literature to calculate the potential role of genetic polymorphisms in reducing reaction rates of the CYP450s and phenotypic variability in the abundance of individual CYP450s. Value of information analysis was used to estimate the relative contributions of different sources of uncertainty in the CYP450 metabolism of CP. The analysis indicates that uncertainty about the phenotypic variability in the abundance of the individual CYP450s is likely to be larger than variability due to genetic polymorphisms of CYP450s. This occurs because all four enzymes exert an effect on the rate of CP-oxon production and when a polymorphism occurs in one CYP450 the other enzymes are still effective in producing CP-oxon. Support for this research was from the University of Washington Center for Child Health Risk Research, U.S. Environmental Protection Agency RD-83170901 and National Institute of Environmental Health Sciences P01 ES09601.

1180 ASSESSING THE ADEQUACY OF THE DEFAULT INTERINDIVIDUAL VARIABILITY FACTOR (IVF) USING A PHYSIOLOGICALLY-BASED STEADY-STATE (PBSS) ALGORITHM.

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The pharmacokinetic component of IVF (IVF-PK) used in non-cancer risk assessment corresponds to a default value of 3.16. But the magnitude of IVF-PK as a function of specific subpopulations is not known for several chemicals. The present study used a PBSS algorithm to characterize the magnitude of IVF-PK on the basis of internal dose (blood concentration (C_{blood}) and rate of metabolite produced/L liver (RAM)) for dichloromethane, tetrachloroethylene, 1,4-dioxane, toluene, styrene, xylene, carbon tetrachloride, ethylbenzene, benzene, chloroform, trichloroethylene and vinyl chloride. Solving the algorithm required data on body weight, liver blood flow, alveolar ventilation rate, liver volume and hepatic content of CYP2E1. The probability distributions for these parameters were obtained from P^3M software and the published literature for seven subgroups of human population: neonates (0-30 days), newborns (30 days – 4 years), children and adolescents (5-19 years), adults (20-44 years and 45-64 years), elderly (65-90 years) and pregnant women. Monte Carlo simulations were performed for each of the dose surrogates, and the ratio of the 95th percentile value for each subgroup over the 50th percentile value in adults was computed. The resulting IVF-PK based on C_{blood} varied from 0.6 to 7.04 depending of the subgroup and substance. The results for C_{blood} indicate that pregnant women, followed by neonates, represented the subpopulation with the highest mean value for IVF-PK (2.79 and 2.54, respectively) for these 12 chemicals. For RAM, the elderly and adults aged > 45 years had the highest mean value of IVF-PK (1.7), while newborns and pregnant women had the lowest values (1.02 and 1.05, respectively). Overall, the present study successfully used a mechanistic algorithm to characterize the sensitive sub-populations with respect to specific dose surrogates and the adequacy of the currently used default value of IVF-PK for oral exposure

1181 TUMOUR DATA FROM HAN WISTAR RAT DIETARY AND ORAL GAVAGE TUMORIGENICITY STUDIES, COMPLETED OVER THE PERIOD OF 1994 TO 2006.

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Previous reviews of Han Wistar (HsdBrl:WH) rat tumorigenicity studies, completed over 1994 to 2000, indicated that the strain had a high survival rate, a satisfactory tumour profile not masked by geriatric lesions and was therefore a suitable strain for use in tumorigenicity studies. A further review of up to 35 studies, completed up to 2006, of the mortality and in-life data confirmed that the strain continues with low mortality at 2 years (mean±SD: 24±6.5%, males and 32±8.8%, females) with no major differences over time or between the dietary and oral gavage routes of administration. Partnering the latter review, the purpose of this study was to establish if the tumour profile had changed over time and also if there were any differences between these routes of administration. The most prevalent tumours continue to be pituitary adenoma in males (>28%) and females (>56%), and mammary fibroadenoma in females (>22%). These tumours (also the major pathological factors contributory to death) did not show any notable differences over time (1994-99 verses 2000-06) or between the routes of administration. There were some minor differences for the less prevalent tumours (<16%), for example, lower incidences in the second time period for mesenteric lymph node (MLN) haemangioma (both sexes), and thymus thymoma and uterine polyps in females. Compared with dietary studies, oral gavage studies showed higher incidences of MLN haemangioma (both sexes), adrenal phaeochromocytoma (males) and, in females, adrenal cortical and thyroid c-cell adenoma. The results indicate that the Han Wistar rat has a well-defined tumour profile with relatively low incidences of tumours, with only minor differences over time or between the routes of administration. It can, therefore, be concluded that the Han Wistar rat continues to be a suitable strain for use in tumorigenicity studies, particularly in view of the regulatory requirement to reach 2 years with an adequate number of survivors.

1182 RAT STRAIN DIFFERENCES IN NASAL TISSUE RESPONSE AND METABOLISM.

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While there are extensive investigations of species differences in toxic response, surveys of intra-strain differences in tissues other than liver are lacking, yet such information has implications for risk assessment. Literature reviews of cytogenic and tumor responses in the nasal regions of rat strains were carried out for naphthalene, propylene oxide and formaldehyde to aid in determining the range and relevance of nasal responses in these strains for assessing cancer risk to humans. This review of available data from studies employing Sprague Dawley (SD), Fisher 344 (F344), Wistar (WT), and other rat strains, allowed for a diagrammatic mapping and comparison of abnormal cellular responses and key enzymes involved in metabolism. The distributions of these responses at various test concentrations and durations are presented and critical data gaps are highlighted. Bioactivation by CYP is associated with nasal injury / toxicity; olfactory nasal regions show highest P450 concentrations whereas non-olfactory regions have 50 times lower CYP metabolic activity. Detoxifying pathways include glutathione-S-transferase (GST) & epoxide hydrolase (EH); in Long Evans rats, EH is not reported to be localized in the Dorsal Meatus, a nasal area associated with injury for some chemicals. Distribution of metabolic enzymes rather than absolute level of an enzyme may dictate lesion distribution for bioactivated toxicants. However, while differences in metabolism have been documented between SD, F344, and WT in tissues such as liver or lung, those for nasal tissue still require further research.

1183 DOSE-RESPONSE COMPARISONS OF SINGLE *VS.* REPEATED EXPOSURES IN DEVELOPMENTAL TOXICITY STUDIES.

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Developmental studies typically utilize repeated exposures either throughout gestation or during specific periods during gestation, particularly organogenesis. Historically, developmental endpoints have been used in chronic (lifetime), but not acute (single exposure) risk assessments. Some developmental toxicants, however, may produce toxicity in the fetus from a single exposure, presumably during specific developmental windows of susceptibility, and would thus represent an acute exposure. Information available on single vs. repeated exposure developmental studies for the same agent was gathered and analyzed. A catalog was developed of 133 studies on 58 agents including phthalates, metals, and pesticides. The level and kinds of responses observed for single vs. repeated day exposures were evaluated to determine the degree of similarity and ability to predict the single exposure response based on the repeated exposure response. Benchmark dose analyses were performed on selected subsets of chemicals (butyl benzyl phthalate and tri-butyl tin) to provide a quantitative measure for comparing dose-responses. Preliminary results for embryolethality indicated that the single exposures required a 2- to 4-fold higher dose than the repeated exposures to produce the same level of response. For certain malformations, the single exposures produced similar levels of response at doses that were close to those seen in the repeated exposures. Appreciable differences in biological half-life (and corresponding dose metrics) as well as specific windows of susceptibility may partially explain the observed acute vs. repeated exposure dose-response relationships. Our preliminary results, however, highlight the

importance of a more thorough evaluation of results from repeated dose developmental toxicity studies for relevance to both chronic and acute risk assessments and for better understanding of the actual dose needed to produce the observed response if the effect is associated with a specific window of susceptibility.

1184 RETROSPECTIVE ANALYSIS OF MULTIGENERATION REPRODUCTION TOXICITY STUDIES: WHAT IS THE IMPACT OF THE SECOND GENERATION IN HAZARD ASSESSMENT FOR PESTICIDES?

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Pesticides go through an extensive evaluation before entering the US market. This evaluation consists of a comprehensive assessment of the toxicological profile of the pesticide including the potential to cause reproductive toxic effects. As interest mounts on reducing the number of animals used in toxicology studies, the US EPA's Office of Pesticide Programs (OPP), the National Heath and Environmental Effects Research Laboratory (NHEERL), and the National Center for Computational Toxicology (NCCT) conducted a retrospective analysis of the multigeneration reproduction toxicity database to ascertain the contribution of the second generation to hazard identification and characterization. A relational database designed by NCCT was used for the analysis. Topics evaluated included whether or not unique effects or lower lowest-observed-adverse-effect levels (LOAELs) were identified in the F2 generation relative to the F1 as well as the range of toxic effects identified in the study.

In this analysis over a 100 pesticides were evaluated encompassing a wide array of chemical classes including organophosphates, pyrethroids, carbamates, conazoles, and known reproductive and endocrine toxicants. For the vast majority of the chemicals, the effects identified in the F2 offspring were similar or less severe than those identified in the F1 generation and the F1 generation LOAELs were ≤ the F2 generation LOAELs. Thus, this analysis indicates that the F2 generation had little impact on risk assessments for NOAEL/LOAEL and endpoint selection or on FQPA safety factor decisions, and corroborates the conclusions described by Cooper et al (Crit. Rev. Toxicol. (2006) 36(1):69-98) and Janer et al. (Repro. Toxicol. (2007) 24(1):97-102).

This abstract does not necessarily reflect the views or policy of the US EPA.

1185 THE IMPORTANCE OF ASBESTOS FIBER LENGTH AS A PREDICTOR OF POTENCY FOR ASBESTOS-RELATED DISEASE.

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Differences in chemical and crystalline composition, fiber dimensions, aerodynamic characteristics and biodurability are among the critical factors that define the toxicological and pathological consequences of asbestos exposure. Specifically, fiber length impacts whether the fiber is respired, whether and how deeply it is deposited in the lung and how efficiently and rapidly it may be cleared. Research investigating how fiber length affects the potency of asbestos and non-asbestos fiber types has been conducted since the early 1970s. However, interpretation of the data collected to date has resulted in some degree of disagreement within the scientific community on whether fiber length is an accurate predictor of fiber potency. We have collected, reviewed and evaluated a complete set of the peer-reviewed literature on this topic and have assembled an era-specific analysis of what was known historically and contemporaneously about fiber length as a predictor of asbestos-related health effects. We have also provided a thorough discussion of the studies offered as support by those in opposition of the 'short-fiber theory.' Based on our analyses, we believe that the data presented in the peer-reviewed literature over the course of the last three decades support the conclusions that exposure to fibers longer than 10 µm and perhaps 20 µm are required to significantly increase the risk of developing asbestos-related disease and that there is very little, if any, risk associated with exposure to those fibers shorter than 5 µm. Fiber length as a predictor of potency has begun to be evaluated by several federal agencies in the U.S. and could significantly influence future asbestos regulatory decisions.

1186 SKIN SENSITIZATION TO HAIR DYE ACTIVES: USE OF SKIN PENETRATION DATA IN A QUANTITATIVE RISK ASSESSMENT APPROACH.

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Consumer allergy to hair dye actives is an important topic for the safety of hair dye products. QRA (quantitative risk assessment) includes the detailed knowledge of the allergenic potency and the dose response characteristics of the ingredient as well as the assessment of the consumer exposure. We introduce an approach for estimating the skin exposure to hair dye actives. Specifically, we suggest to assess the consumer exposure level based on exposure data available from skin penetration studies. These in vitro studies (OECD guideline 428) provide direct quantification of the concentrations found on and in the skin, i.e. the actual consumer exposure level (CEL) at a distinct use concentration under realistic use conditions. For example, the measured CEL for p-phenylenediamine was 22 µg/cm2, determined after application of a hair dye product under use condition (30 min exposure time and rinsing with water and shampoo). In dose response experiments, we found a linear relationship between the applied amount and the measured CELs. We conclude that this approach allows for a more robust and accurate quantitative risk assessment for existing hair dye actives as well as new actives being developed to improve the safety profile of hair dyes.

1187 A NOVEL FRAMEWORK FOR ASSESSING RISK FROM FORMALDEHYDE-RELEASING BIOCIDES.

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A number of biocides release formaldehyde (FA) under specific use conditions, leading to concerns of additional human risk beyond that attributed to the parent molecule. The biocidal activity of formaldehyde releasers may not be solely based on the released formaldehyde. The formaldehyde releaser, under use conditions, forms an equilibrium with the carrier molecule and formaldehyde. Traditional risk evaluations for FA exposure utilize a linearized multistage model to derive the hazard endpoint and risk assessments typically use conservative exposure models, both of which likely lead to overestimating risk. We have developed a novel risk assessment approach for product registration of biocidal active ingredients under the European Union Biocidal Products Directive (98/8/EC; BPD) that evaluates concurrent risks from exposure to both the parent molecules and FA. The approach includes: 1) examining the toxicity inherent to the parent molecules, 2) use of a threshold approach for FA as developed by the German Institute for Risk Assessment (BfR), 3) use of conservative assumptions with regard to the occurrence of both the biocide and FA, and 4) use of emission chamber data in conjunction with two-compartment numerical integration models of exposure. Toxicity testing of the biocides demonstrated little target organ toxicity other than point of contact irritation, similar to that observed for FA.

Chamber emission data in conjunction with a numerical integration model determined that exposure to FA during painting was less than 0.1 ppm in air for most scenarios. We believe this approach is protective yet more realistic than traditional FA exposure estimations and application of the numerical integration model allows increased flexibility in the evaluation of emission data for consumer and worker exposure scenarios.

1188 EVALUATING THE TOXICOLOGICAL SIGNIFICANCE OF ENDPOINTS FROM HUMAN AND ANIMAL STUDIES: USING PERFLUORINATED COMPOUNDS (PFCS) AS AN EXAMPLE.

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Adverse health effects (or their immediate precursors) form the basis for risk-based criteria. However, given the increased amount of mechanistic data available on subcellular biochemical changes, it is important to recognize that not all changes observed in such studies are adverse. When evaluating toxicological significance, it is important to consider issues such as dose response, reversibility, functional impairment, human relevance, and whether the effect is a disease precursor. We examined the extensive toxicological database of the PFCs perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) in the context of evaluating the toxicological significance of effects observed in human and animal studies. Of the key animals studies, we have concluded that many of the effects identified as sensitive endpoints, such as alterations in thyroid hormone, cholesterol, and ALT levels, are not adverse and hence not appropriate for use in criteria development. In several cases, these endpoints did not show a dose-response relationship, were within normal ranges, were not associated with any physiological deficits. Our evaluation of human studies in PFC workers leads to similar conclusions; the isolated observed changes related to blood chemistry levels were within typical levels, and were not associated with increased disease. We also note that recent human developmental studies have reported an association between PFC exposure and reduced birth weight and head circumference, but changes are small, within normal ranges, and of uncertain toxicological significance. Overall, we conclude that some of the observed changes in blood chemistry levels, as well as developmental findings in recent human studies, are not appropriate endpoints on which to base toxicity criteria. The findings of no significant effects in occupational studies, as well as the more

significant changes observed in animals studies (e.g., relative liver weight in monkeys and overt developmental effects in mice) are more suitable for developing risk-based criteria for PFCs.

1189 CASE STUDIES FOR THE DEVELOPMENT OF A PATHOPHYSIOLOGICAL PROGRESSION MODEL.

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U.S. EPA's National Center for Environmental Assessment is developing approaches to identify/categorize toxicological endpoints along a pathophysiological continuum, using the framework presented by Schulte and colleagues (exposure, internal dose, biologically effective dose, early biological effect, altered structure/function, and clinical disease). Information on the steps in biological pathways can be used to help evaluate adversity and biological significance of precursor effects, and to better inform the use of toxicological endpoints in human health risk assessments. Two case studies – fatty liver (steatosis) resulting from exposure to carbon tetrachloride and pulmonary fibrosis resulting from exposure to phosgene – have been developed. Steatosis is an accumulation of triglycerides in hepatocytes and occurs when the rate of import or synthesis of fatty acids by hepatocytes exceeds the rate of export or catabolism. Key events in carbon tetrachloride-induced steatosis include metabolism to free radicals; covalent bonding to cellular molecules; and reduction in the level of very low-density lipoproteins, thereby inhibiting hepatocyte secretion of triglycerides from the liver. Steatosis can sensitize the liver to further injury such as inflammation, fibrosis, and cirrhosis. Key events in the development of phosgene-induced fibrosis include oxidative stress (GSH depletion); inflammation of bronchial and alveolar structures; disruption of the blood:air barrier; pulmonary edema; inflammation, including release of cytokines; neutrophil and alveolar type II cell activation/migration; accumulation/polymerization of fibrin-rich material and collagen; activation of the coagulation cascade; and dysregulated repair. The diversity of endpoints observed in this continuum may reflect differences in the sensitivities to phosgene across species. For both case studies, biomarkers of exposure and effect, supporting evidence, and human relevance are discussed.

1190 REMOVING CENSORSHIP BIAS FROM CORRELATION ESTIMATES OF DATA SETS CENSORED BY LIMITS OF DETECTION.

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Correlations can be used to infer causal mechanisms between exposure metrics (e.g. pesticides) and resulting impacts (e.g. metabolites). However, sensitivity limitations in analytical laboratory methods often result in samples that fall below the limits of detection (LOD). Limiting the analysis to data with values above the LOD will induce a bias in the resulting correlation estimates, as will artificially substituting values for the unobserved censored data. Correlation biases can alter our inference of causal mechanisms as well as alter the strength of the dose-response relationships. Censorship bias can be eliminated by representing the censored data as draws from the overall multivariate distribution, but limited to the range below the LOD. Bayesian Markov chain Monte Carlo (MCMC) methods provide a robust approach for analyzing datasets of this type. These methods can easily handle multiple LOD cutoffs, even within the same laboratory procedure. Examples from organophosphate pesticides (OP) and resulting metabolites are used to demonstrate how existing methods induce censorship bias and how Bayesian MCMC methods can remove this bias. [Support for this research was from the University of Washington Center for Child Health Risk Research, U.S. Environmental Protection Agency RD-83170901 and National Institute of Environmental Health Sciences 5 P01 ES09601.]

1191 THE POWER TO DETECT A DIFFERENCE: DETERMINING SAMPLE SIZE REQUIREMENTS FOR EVALUATION OF REPRODUCTIVE/DEVELOPMENTAL EFFECTS FROM EXPOSURE TO COMPLEX MIXTURES OF DISINFECTION BYPRODUCTS.

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Toxicological assessment of environmentally realistic complex mixtures of drinking-water disinfection byproducts (DBPs) are needed to address concerns raised by some epidemiological studies showing associations between exposure to chemically disinfected water and adverse reproductive and developmental outcomes. There is a growing emphasis on inclusion of low, environmentally relevant doses when assessing complex chemical mixtures. However, such environmentally relevant doses, which are typically in the low response region, may cause effects that would not be readily detected in standard animal testing protocols using typical sample sizes, as these assays were developed to have appropriate power for much higher dose levels. Proper study design, in particular the characterization of statistical power, is critical to ensuring that experimental results are meaningful at low levels and to proper interpretation of negative data. As standard methods were not appropriate, a novel statistical approach to calculating power was developed for the multigenerational rodent bioassay included in the third phase of the U.S. EPA's 4Lab Project. The bioassay was intended for assessment of reproductive and developmental effects from exposure to a complex mixture of DBPs in drinking water concentrates. Using data generated in earlier phases of the 4Lab Project and the innovative approach, the power calculation results show that a study design of two blocks of 100 animals each with a 40:60 ratio of control:treated animals and a significance level of 0.05 provides approximately 90% and 45% power to detect pup weight decreases and prenatal loss increases, respectively. In conclusion, power calculation methods were developed, then used to improve the design of a multigenerational reproductive/developmental study with a complex mixture of DBPs. (This abstract does not reflect EPA policy.)

1192 GEOSPATIAL CONSIDERATIONS IN CALCULATING 95% UPPER CONFIDENCE LIMITS ON THE MEAN.

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The upper 95% confidence limit on the mean (95% UCL) is often used as an upper-bound estimate of average contaminant concentration for risk assessments at hazardous waste sites. Mathematical tools available from EPA (PROUCL v4) developed to simplify the computation of 95% UCLs have gained broad acceptance and use in environmental decision-making. However, a fundamental assumption in the development of 95% UCLs is random sampling - a condition rarely encountered in real-world datasets. Typically, sites are assessed in a phased approach which targets sampling toward areas with known or suspected elevated concentrations. The biased dataset that results often over-represents the most heavily contaminated portions of the site. An alternative method for developing 95% UCLs is proposed that accounts for non-random sampling (spatial UCL). Theissen polygons are constructed for sample datasets such that the polygon's boundaries define the area that is closest to each sample location relative to all other locations. Area weighting factors are generated for each polygon relative to the area of the exposure unit. Bootstrap resampling techniques are then applied to the weighted sample data. To test the effectiveness of this method, random response surfaces were generated with known concentration parameters (true mean is known). Biased and random sampling locations were selected for each response surface. Results from traditional 95% UCL methods and spatial UCL techniques were computed for each sample set. Results show that using EPA methods, non-random sampling results in an over-estimation of 95% UCLs - an effect that is exacerbated by increasing the sample size. Spatial UCL techniques result in UCLs closer to the true mean with increasing efficiency with increasing sample size. The performance of spatial UCL techniques suggests that consideration should be given to the assumption of random sampling in estimating exposure point concentrations for risk assessment.

1193 ASSESSMENT OF THE RELEVANCE OF CARBOXIN SOIL METABOLITES.

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Two soil metabolites of carboxin, (5,6-dihydro-2-methyl-N-phenyl-1,4-oxathiin-3-carboxamide), were detected in both an aerobic soil metabolism study and a field dissipation study. These metabolites, P/V-54 (oxathiine amide sulfoxide) and P/V-55 (oxathiine amide sulfone), appear to be unique to soil and have not been identified in rat metabolism studies. The metabolites were evaluated by FOCUS PELMO (3.3.2) modeling and determined to have the potential to exceed 0.1µg/l in groundwater. In order to obtain listing of carboxin on Annex I, non-relevance of metabolites P/V-54 and P/V-55 has to be established, therefore an assessment was conducted in accordance with the Guidance Document on the Assessment of the Relevance of Metabolites in Groundwater of Substances Regulated under Council Directive 91/414/EEC (Sanco/221/2000 – rev. 10, 25 February 2003). The assessment included screening for biological activity, SAR modeling, acute and subchronic mammalian studies, ecotoxicological studies and mutagenicity studies. On the basis of close structural similarity between the two metabolites, studies were only conducted with P/V-54. The assessment concluded that the soil metabolites

P/V-54 and P/V-55 are not of toxicological concern, and pose a negligible risk to man. Therefore, P/V-54 and P/V-55 are considered to be non-relevant metabolites of carboxin.

1194 BIOAVAILABILITY ADJUSTMENT OF RISK-BASED SOIL CLEANUP LEVELS.

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Majority of risk-based soil cleanup levels are currently based on total contaminant concentrations assuming that they are 100% bioavaible. In most instances, the assumption of 100% bioavailability is conservative because most toxicity tests utilize forms of a chemical that tend to be readily absorbed. However, it is generally recognized that the rate of absorption of a chemical of highly insoluble forms existing in soil is much lower than that of more soluble forms via oral route. Furthermore, there is growing evidence that some inorganic and organic chemicals, such as metals, pesticides, and polycyclic aromatic hydrocarbons (PAHs), undergo an aging process in soil whereby the contaminants are sequestered in the soil so as to further decrease their bioavailability and toxicity to biological receptors. They are therefore less available than it is suggested by their total concentrations, such that cleanup levels expressed as bulk concentrations may not correlate with actual risk. This paper presents a case study of a railroad site where elevated arsenic levels are broadly distributed across a majority of the site, resulting from the possible historical use of arsenic-laced slag as a structural base for the railroad tracks. In addition to arsenic, other contaminants include PAHs, lead, mercury, and PCBs. These contaminants are not subject to significant volatilization, leaching, or degradation and thereby persistent in soil. The immobility and of these contaminates are supported by their limited vertical distribution in soil and the lack of presence in groundwater. Because of inaccessibility, these chemicals are unlikely to cause harm to biological receptors than is suggested by their total concentrations. An in vitro bioavailability and chemical speciation study is used to quantify relative bioavaibility of these contaminants. The results are integrated into risk calculation to characterize a more representative estimate of risks.

1195 BIOREACTIVITY OF LEACHATE FROM MUNICIPAL SOLID WASTE LANDFILLS — ASSESSMENT OF TOXICITY.

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There are strong national and international concerns about the possible adverse health effects of living in the vicinity of municipal waste landfills. An understanding of the ranges of toxicity of landfill emissions is crucial to determine the degree of concern we should have about the potential effects they could have upon nearby populations and the surrounding environment.

Leachates from three different types of municipal waste landfills were collected every month for a year, and screened for their potential to induce in vitro toxicity. Leachate was sampled from: 1) active & partially unlined; 2) active & dilute and disperse; 3) containment & restored landfills. Bioreactivity was measured by a plasmid DNA scission assay (PSA), and 2′,7′-dichlorodihydrofluorescin dye fluorescence (DCFH). The PSA and DCFH assays have been used by previous investigators to measure the potential for reactive oxygen species(ROS). The concentration of leachate to produce 50% DNA damage (TD50) was assessed for seasonal and landfill-specific trends. The effect of incubating leachates with metal chelators (EDTA, Desferoxamine mesylate) was also investigated.

The results show that landfill leachates cause damage to plasmid DNA in a dose-dependent manner, with leachate from the restored landfill shown to be as toxic as active landfill leachate. Leachate toxicity was significantly ameliorated by metal chelation, which may indicate metal ions as a mediator for ROS. A statistically significant correlation between the PSA TD50 values and the DCFH assay was found (Spearman's coefficient –0.723). Toxicity was shown to vary between different types of landfills, with evidence of site-specific trends. The novel use of the DCFH assay with environmental effluent samples in this study merits further investigation as an indicator of bioreactivity. The PSA and DCFH assay have shown a possible link between MSW leachates and generation of ROS.

1196 XENOBIOTIC METABOLISM RESEARCH AND ITS APPLICATION TO HUMAN AND ECOLOGICAL EXPOSURE AND RISK ASSESSMENT.

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A major uncertainty in risk assessment is determining the exposure of a target organism to a chemical stressor, and a confounding factor is the transformation of the chemical to a toxic metabolite inside the target organism. Physiologically-based pharmacokinetic (PBPK) models are the preferred tool to describe the fate of xenobiotics in physiological systems, since these models can be used to evaluate tissue dose relative to route of uptake. A major obstacle to fully implementing PBPK models for risk assessment is the lack of xenobiotic metabolism data for a majority of chemicals in use. This data gap becomes a greater obstacle when risk is extrapolated across species such as from rat to human or to an ecologically important organism. To address this, we develop and apply innovative techniques for elucidating the kinetics and mechanisms of xenobiotic metabolism, and apply these techniques to the understanding and modeling of chemical exposure for informing human health and ecological risk assessment. Unique to our program, concomitant substrate depletion and metabolite formation kinetic parameters (i.e., Vmax and KM) are determined using non-linear models. Specific enzyme inhibitors, purified recombinant human cytochrome P450s (CYPs), stable isotopes, stereoselectivity and molecular docking are used to elucidate the mechanisms of xenobiotic metabolism. Kinetic parameters and mechanistic information are integrated to develop PBPK models, which are then validated in vivo. The entire paradigm, from metabolite map elucidation to PBPK model development, is applied across multiple species (e.g., human, rodent, fish, crustacean and insect) to develop and test cross-species extrapolations for risk assessment. We will illustrate this paradigm and its application to risk assessment using results from 20 conazole fungicides and the piscicide antimycin A.

1197 BIOMONITORING EQUIVALENTS: CASE STUDIES.

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To place human biomonitoring results into a risk context, the Biomonitoring Equivalents (BE) Pilot Project explored the feasibility and technical challenges of integrating chemical-specific pharmacokinetic data with existing assessments of tolerable exposures (e.g., reference doses, reference concentrations, tolerable daily intakes, minimal risk levels, etc.) to estimate concentrations of environmental chemicals in biological media that are consistent with exposure guidance values. Four chemical case studies were included in the pilot project: acrylamide, 2,4-D, cadmium, and toluene. These chemicals present a range of technical and toxicological challenges which provide opportunities to explore different approaches to BE derivation. BE values for blood concentration of toluene were derived based on available pharmacokinetic data and existing exposure guidance values, while both urinary and blood or plasma BE values were derived for cadmium, acrylamide, and 2,4-D. Approaches to both cancer-based and non-cancer-based exposure guidance values are demonstrated. Several types of pharmacokinetic data and models are used in the BE value derivations, including simple data sets correlating dose with blood concentration, one compartment pharmacokinetic models, and fully-developed PBPK models. Urinary BE values are generally derived assuming steady-state exposure using a mass-balance approach. The resulting BE values can be used to assess the measured concentrations in population-based biomonitoring studies for screening level risk appraisals and these can be used to prioritize actions to more fully evaluate potential sources, exposures and dosimetry for risk assessments and, where warranted, for risk management activities.

1198 A FRAMEWORK FOR EVALUATING SERUM DIOXIN DATA DERIVED FROM BIOMONITORING STUDIES.

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Human biomonitoring studies have been conducted for various chemicals over the last century and are increasingly being used to determine potential exposures to chemicals. An important component of such characterizations is the comparison of measured levels to those in a relevant reference population. Dioxin-like compounds (DLCs) have been the focus of a number of recent biomonitoring studies and present unique challenges. A transparent, multi-step decision framework was developed for use in determining whether measured DLC levels are similar to or different from a reference population. The initial step of the framework involves calculating the lipid-adjusted dioxin toxic equivalency serum concentrations (TEQ) for individuals in the study population. Next, lipid-adjusted TEQs, adjusted for age, race, and gender, are developed for a relevant reference population. In the absence of a study-specific reference population, sampling data from the National Health and Nutrition Examination Surveys can be used to develop background reference values. Each individual's TEQ is then compared to the 95th percentile TEQ value of the reference population. If individuals are found to have TEQs above the 95th percentile benchmark, a statistical test of proportions is used to evaluate whether the study population as a whole may have experienced exposures beyond that of the reference population. Additionally, exposure histories should be reviewed and con-

gener fingerprint analyses should be conducted to determine if other potential sources of exposure are impacting the TEQ measurements in those individuals above the 95th percentile. If individuals are determined to be outliers based on these additional evaluations, then these individuals can be removed from the study population and the statistical test of proportions can be re-run. Given the complexities and uncertainties associated with using biomonitoring data in exposure assessments, this framework provides a useful and comprehensive tool for evaluating such data.

1199 BIOMONITORING EQUIVALENTS: TECHNICAL ISSUES AND GUIDELINES – REPORT FROM THE EXPERT WORKSHOP.

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To place human biomonitoring results into a risk context, the Biomonitoring Equivalents (BE) Pilot Project explored the feasibility and technical challenges of integrating chemical-specific pharmacokinetic data with existing assessments of tolerable exposure (reference doses, reference concentrations, tolerable daily intakes, minimal risk levels, etc.) to estimate concentrations of environmental chemicals in biological media that are consistent with exposure guidance values. An expert panel including scientists from academia, government, and industry was convened to examine issues related to derivation of BE values. The BE Derivation panel examined issues related to selection and use of biological media and analytes, application of animal pharmacokinetic data to point of departure dosing regimens, inter- and intra-species variability and the impact on estimated BE values. The BE Derivation panel also examined issues associated with interpretation of biomarkers for short-lived compounds and the use of urinary markers including methods of accounting for hydration status. The panel recommended that the point of departure underlying exposure guidance values such as reference doses be the focus of derivation where data make this feasible. Depending on the understanding of relevant internal dose metrics and the relationship to the measured biomarker, some components of default uncertainty factors may be replaced with modeling or with the variability observed in the biomarker measurements. Recommendations of the panel for derivation approaches and outstanding issues that need additional effort will be presented.

1200 COMPARISON OF HUMAN CYP INDUCTION BETWEEN IN VIVO AND IN VITRO STUDIES USING PXB MICE® WITH HIGHLY REPOPULATED HUMANIZED LIVER.

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In vitro enzyme induction studies using human hepatocytes have become a standard procedure in drug development. However there are some difficulties in the prediction of the effects in humans, because of the differences in exposure profiles between *in vitro*-and *in vivo*-test systems.
In the present study, we investigated human (h) CYP1A2 and 3A4 enzyme induction in PXB mouse liver (*in vivo* study) as well as in human hepatocytes isolated from the PXB mice (*in vitro* study) using the same donor hepatocytes for both studies. For the *in vivo* study, PXB mice were administered Rifampicin and 3-MC once a day for 4 days with doses of 2 and 20 mg/kg for Rifampicin and, 0.1 and 10 mg/kg for 3-MC. For the *in vitro* study, human hepatocytes from PXB mice were treated with 10, 50, and 250 ng/mL of 3-MC, or 5 and 25 µg/mL of Rifampicin for 24 h and 48 h at 37°C in a 5% CO_2 incubator. These low dose levels for the *in vitro* study were based on the plasma concentration levels observed in the *in vivo* study.
In the *in vivo* study, hCYP1A2 mRNA expression level significantly increased (2-fold) in the 20 mg/kg of 3-MC group. The AUCs lowered significantly at Day 3 in both the 2 mg/kg and 20 mg/kg groups when compared to those observed at Day 0. Treatment with 10 mg/kg of Rifampicin significantly increased the hCYP3A4 mRNA expression level (8-fold) in the PXB mouse liver. The AUCs lowered significantly at Day 3 in both of 0.1 and 10 mg/kg groups when compared to those observed at Day 0. The *in vitro* study revealed that hCYP1A2 and hCYP3A4 mRNAs were significantly induced at the same concentrations to Cmax levels as in the *in vivo* study (10 ng/mL for 3MC and 5 µg/mL for Rifampicin).
Since our PXB mice provide the same donor hepatocytes for both *in vivo* and *in vitro* test systems, the results from this study offer an exemplary case for the discussion of the comparability between *in vivo* and *in vitro* induction effects based on the exposure profile.

1201 DEVELOPMENT AND VALIDATION OF A HEPATOCYTE ASSAY THAT DETECTS REACTIVE METABOLITE CYTOTOXICITY.

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Cytotoxic reactive metabolites (CRM) play a key role in drug-induced hepatotoxicity, especially for idiosyncratic hepatotoxicity. In our laboratories, a reactive metabolite cytotoxicity assay (RMCA) was developed using fresh (1°) and cryopreserved (cryo) primary rat hepatocytes (heps) based on the working hypothesis that if CRM are formed from a parent toxicant, depletion of reduced glutathione (GSH) would lead to increased cytotoxicity in the heps. The 1° heps, isolated from male Sprague-Dawley rats where compared to pooled rat cryo heps. The heps were treated with buthionine sulfoximide (BSO), an inhibitor of GSH biosynthesis, to reduce cellular GSH pools. Time- and dose-dependent decreases in GSH in response to BSO were observed in both 1° and cyro heps. BSO treatments were optimized to deplete GSH to < 50% of controls where no apparent cytotoxicity (based on cellular ATP content) was observed. The heps were treated with prototype hepatotoxicants that are known to form CRM upon hepatic metabolism. The cryo heps contained less initial GSH and were more sensitive to BSO treatment than 1° heps. In 1° heps, acetaminophen and cyclophosphamide were consistently more cytotoxic in the BSO-treated than the untreated cells. In contrast, BSO-treated cryo heps were not as sensitive to acetaminophen as the 1° heps, most likely due to reduced metabolism in cryo heps. The human idiosyncratic hepatotoxicants tacrine and valporic acid did not produce BSO-induced cytotoxicity in either hep source, which may be due to a differential in rat-human hepatic metabolism or reduced metabolic capacity in culture. These two possibilities are being explored further. The RMCA is now being extended to human hepatocytes in our laboratories to examine possible species specific differential response. The RMCA is proposed to be used as a screening assay in early drug development to identify compounds that would form CRM upon hepatic metabolism.

1202 FUNCTIONAL STABILITY OF HUMAN HEPATOMA HEPARG CELLS AND THEIR USE FOR CHRONIC TOXICITY AND GENOTOXICITY STUDIES.

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Presently there is no pertinent in vitro cell model for the evaluation of hepatic chronic toxicity and genotoxicity of chemicals in humans. HepaRG cells represent the only human hepatoma cell line capable of metabolizing chemicals at levels comparable to those measured in primary human hepatocyte cultures (Aninat C et al, Drug Metabol. Dispos., 2006, 34,75-83).
We show that these cells in addition to the major cytochromes P450 (CYP1A2, 2C9, 2E1, 3A4,..) and nuclear receptors such as CAR, PXR and PPAR, have retained high levels of phase 2 enzymes and antioxidant enzymes as well as expression of membrane transporters. These functions, including responsiveness of CYPs to prototypical inducers, are well preserved at least during passages 7 to 17 and are stable when cells are maintained differentiated at confluency for one month or more. HepaRG cells were used to demonstrate dose-dependent cumulative toxicity as well genotoxicity (Comet assay) of aflatoxin B1.
Our results report the first evidence that HepaRG cells could represent a suitable in vitro liver cell model for the analysis of acute and chronic toxicity as well genotoxicity of chemicals in human liver.

1203 APPLICATION OF A NEW HEPATOCYTES PRESERVATION TECHNOLOGY USING A UNIQUE MATRIX TO *IN VITRO* TOXICOLOGY ASSAYS.

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Cryopreserved hepatocytes are well used in assessment of hepatotoxic potential of new drug candidates. Since their metabolic activities are compromised, there are a lot of cases in which reactive metabolite-mediated toxicity could not be evaluated precisely. Recently, Abcellute Ltd. has developed a new hepatocytes preservation

technology using a unique matrix. We examined the quality of rat and canine hepatocytes preserved up to 21 days and 7 days, respectively, after preserving the cells by Abcellute's method and evaluated the sensitivity to hepatotoxicants such as aflatoxin B1 (AFB1) and cyclophosphamide (CP) with these preserved hepatocytes.
Up to 4 days after preservation, the following characteristics of preserved rat and canine hepatocytes were comparable to those of freshly isolated ones; viability, ATP content, Phase I and Phase II activity, and sensitivity to hepatotoxicants. Even after 7-day preservation, some CYP activities were retained and CP-induced hepatotocity could be detected in a similar sensitivity to the freshly isolated hepatocytes. Commercially available cryopreserved hepatocytes of rats and dogs exhibited lower ATP content, CYP activity and sensitivity to hepatotoxicants compared to the preserved hepatocytes by Abcellute's method as well as the freshly isolated cells. These results showed that Abcellute's hepatocytes preservation technology had advantages over cryopreserved hepatocytes in in vitro toxicology assays.

1204 CYTOTOXICITY OF DEOXYNIVALENOL IN RAT LIVER CLONE-9 CELLS IN CULTURE.

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Deoxynivalenol (DON) is a mycotoxin food contaminant found in several cereal grains. The literature on the liver toxicity of DON in vivo is conflicting and does not clearly characterize its hepatotoxic effects. Cultured rat liver clone-9 cells were used as a model to assess the hepatotoxic potential of DON. The cell cultures, seeded onto 96-well plates, were treated at confluence with varying concentrations of DON (0 - 100 µg/ml) for 48 h at 370C in 5% CO2. After the treatment period, the cells were assayed for multiple hepatotoxic endpoints that included cytotoxicity, double-stranded DNA (ds-DNA) content, oxidative stress, and mitochondrial function. Concentration-dependent toxicity of DON, as measured by cytotoxicity and ds-DNA content, was observed over the entire concentration range studied beginning at 0.5µg/ml. DON also induced a significant concentration-dependent increase in oxidative stress at DON concentration above 10 µg/ml. The mitochondrial function of the treated cells was not statistically different from that of the controls. Liver histopathology observed at 3, 24, and 72 hrs following a single intraperitoneal administration of DON (10 mg/kg BW) to adult male rats is consistent with early and mild hepatotoxicity. The overall results of this study suggest that acute DON exposure has early mild cytotoxic effects on hepatocytes in vivo that are expressed as severe effects in rat liver clone-9 cells in vitro.

1205 THE IN VITRO AND IN VIVO FUNCTIONAL EFFECTS OF A LIPOPHILIC ANTI-INFLAMMATORY COMPOUND ON BILIARY CLEARANCE IN THE RAT.

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Several molecules affect bile acid excretion patterns *in vivo* however the mechanisms by which these effects are mediated are not fully understood. We investigated these mechanisms using a lipophilic anti-inflammatory compound from a series of molecules that we have shown to modify biliary excretion patterns (bile flow and bile composition) in the rat. Furthermore, dose levels that produce altered biliary excretion are associated with non-degenerative morphological changes in the hepatobiliary system, including hypertrophy/hyperplasia of the biliary epithelial cells (cholangiocytes).
Using hepatocyte sandwich cultures, we have previously shown that this lipophilic anti-inflammatory compound decreased the efflux of cholyl lysyl fluorescein (CLF) by the bile salt export pump (Bsep), increased the multidrug resistance protein 2 (Mrp2)-mediated efflux of carboxydichlorofluorescein (CDF) and slightly increased the uptake of [^{3}H]-taurocholate via sodium taurocholate co-transporter protein (Ntcp). We have further studied the effects of this compound on biliary function *in vivo*. Rats were dosed with the test compound orally as a single dose over a range of doses or once daily for 7 days at a dose level known to be associated with morphological change in the cholangiocytes. On day 7 following the single dose or on day 8 following completion of the 7 day dose schedule, animals were injected intravenously with CLF and blood was taken at intervals within the 20 minute period post injection. The data showed that there was a significant decrease in plasma elimination of CLF compared to the values from vehicle treated rats. In conclusion, we have shown that this lipophilic anti-inflammatory compound was able to alter biliary transport function *in vitro* and *in vivo*. Furthermore, the doses that altered CLF transport *in vivo* were also associated with morphological changes in biliary epithelial cells. This association may provide important pointers to the mechanism of these morphological changes in the liver.

1206 AN *IN VITRO* SCREENING APPROACH FOR PREDICTING HEPATOTOXICITY.

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Hepatotoxicity is the major cause of commercial drug withdrawal from the market by regulatory agencies. The pharmaceutical industry employs both in vivo and in vitro approaches in an attempt to reduce the rate of attrition during the drug development process. An in vitro approach using immortalized normal human hepatocytes transfected with selected P450's (CYP1A1, CYP3A4, CYP2D6 and CYP2C9) was examined (Pfeifer et al., 1993). The transfected P450's allow detection of intrinsic liver toxicity and also toxicity due to metabolite formation. Control cells (null P450) and P450 transfected cells were treated with known hepatotoxicants (N=110) and non-hepatotoxicants (N=51) to assess the utility of the cell system for identifying agents with potential for human liver toxicity. Cells were treated with compound for 72 hours, with compound re-added every 24 hours. The level of cytotoxicity was measured using a fluorogenic peptide substrate (GF-AFC) specific for intact viable cells. The P450 expressing cells were compared to the null cells to determine if observed toxicity was P450 metabolism dependent. Student's t-test was performed as a measure of statisitcal significance. The assay appears suitable as a medium throughput screen for (1) direct acting human hepatotoxicants, (2) compounds requiring metabolism for hepatotoxicity and (3) non-hepatotoxicant agents. This cell system will allow for better decision making and risk assessment with compound development.

1207 SCREENING FOR MECHANISM OF HEPATOTOXICITY: PHOSPHOLIPIDOSIS, STEATOSIS, APOPTOSIS AND INFLAMMATORY MARKERS.

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Sponsor: L. KIng.

Drug-induced hepatic injury is one of the most common reason cited for withdrawal of an approved drug. Hence it is important to have a robust and rapid hepatoxicity screening system. In this study, we were able to develop screening assays to clarify mechanism of hepatotoxicity. All assays were performed using a human hepatic cell line (HepG2). We have included compounds with previously reported hepatotoxic or apoptotic activities or compounds that cause intracellular lipid accumulation. Lipophillic compounds can bind and aggregate phospholipids, forming vesicles within the cells and creating a condition called phospholipidosis. Other compounds can interfere with normal metabolic processes, inhibiting lipid breakdown or stimulating lipogenesis. Apoptosis is a mode of cell death which can be induced by apoptotic compounds. Cells undergoing apoptosis show characteristic biochemical and morphological features including caspase activation. Hepatotoxicity frequently involves inflammation. Therefore, we investigated inflammatory effects of well known hepatotoxic drugs. The secretion of inflammatory markers including IL6, IL8, MCP-1, TNFa and M-SCF was tested in the human hepatic cell line. To measure phospholipidosis, steatosis and apoptosis induction we have used fluorescent labels and IN Cell Analyzer 1000 automated microscope. We have shown that these screening assays provides sensitive and reproducible in vitro system to test for hepatoxicity which previously was only manifested in more complex in vivo models. The in vitro screening allows to select drug scaffolds for development in a cost-effective and high throughput manner.

1208 MICROARRAY ANALYSIS OF TROVAFLOXACIN-INDUCED GENE EXPRESSION CHANGES IN HUMAN HEPATOCYTES COMPARED TO HEPATOCYTES ISOLATED FROM RATS TREATED WITH LPS.

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The fluoroquinolone antibiotic trovafloxacin (TVX) is associated with idiosyncratic hepatotoxicity in humans, whereas other quinolone antibiotics such as levofloxacin (LVX) are not associated with this risk. TVX applied to human hepatocytes (HPCs) induced unique gene expression changes compared with less toxic quinolones and compared with primary rat HPCs treated with TVX. TVX was not hepatotoxic to rats in vivo unless they were pretreated with lipopolysaccharide (LPS) to induce inflammation. We hypothesized that the interspecies differences in gene expression changes in response to TVX exposure in vitro may be due to the isolation of human HPCs from donors who experienced inflammatory stress at the time of death.

Accordingly, we compared TVX-induced gene expression changes in human HPCs and HPCs isolated from rats given LPS in vivo. Rats were treated with LPS (44.4 x 10^6 EU/kg) or vehicle (VEH), then their HPCs were isolated and plated for treatment 4 hr later. HPCs were treated with TVX (400 μM), a pharmacologically equiefficacious concentration of LVX (1 mM) or VEH, then 24 hr later RNA was isolated for microarray analysis. Using LPS/VEH treatment as baseline, the expression profile of TVX-treated HPCs from LPS-treated rats differed substantially from both the TVX-treated human HPCs and LVX-treated HPCs isolated from LPS-treated rats. TVX induced expression changes in 1,516 genes in human HPCs and 4,149 genes in HPCs from rats treated with LPS; 745 of these genes were commonly regulated. The commonly downregulated genes are involved in cytokine and growth factor signaling, whereas the commonly upregulated genes are involved in anti-apoptotic, cell proliferation, and survival pathways. These results suggest the possibility that the cells responded with cell survival signaling to a stress induced by TVX exposure 24hr earlier. Supported by NIH grant GM075865.

1209 GENE EXPRESSION AND PATHWAY ANALYSIS TO STUDY THE EFFECT OF CELLULAR STRESS ON IDIOSYNCRATIC DRUG INJURY IN RAT PRIMARY HEPTOCYTES.

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Several hypotheses, including oxidative stress or reactive drug metabolite(s), have been proposed for causality of idiosyncratic drug-induced liver injury, but direct evidence remains enigmatic. The aim of this study was to develop methods to screen potentially idiosyncratic drug candidates during early drug development. Rat primary hepatocytes were pre-incubated with a cocktail of cytochrome P450 inducers to maintain metabolic capability, and then treated with non-cytotoxic levels of glucose oxidase (stress), as well as with idiosyncratic-causing drugs troglitazone (TRO) and ranitidine (RAN), or their presumably less toxic counter compounds, rosiglitazone (ROS) and cimetidine (CIM). Cytolethality curves for the PPARγ agonists TRO and ROS were clearly left shifted in cells that were pre-disposed to cellular stress. The H2 antagonists RAN and CIM were relatively non-toxic in this model, but predisposition to stress did modestly increase their cytotoxicity. RNA was collected from cells treated with sub-lethal doses of drugs (cytolethality range of 10-25%) and microarray analysis was performed. The largest percent change in overall gene expression ($p<0.02$) was 11% with stress alone, whereas 2-4 % of the genes on the array were significantly perturbed for each drug vs. vehicle control, and for drug+stress vs. stress control. Interestingly, in the absence of stress, TRO and RAN significantly impacted pathways associated with glucose metabolism which were absent for ROS and CIM. Also, there was an induction of oxidative stress pathways by TRO and RAN, but not ROS and CIM, in the absence of stress. Conversely, in the presence of stress, pathway analysis showed no clear differentiating factors between idiosyncratic and non-idiosyncratic drugs. These data suggest that using gene expression profiling and pathway analysis in rat hepatocytes may have value in early detection of compounds at greater risk for idiosyncratic toxicity.

1210 ROLE OF HYDRAZINE IN ISONIAZID-INDUCED HEPATOTOXICITY IN A HEPATOCYTE INFLAMMATION MODEL.

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Isoniazid is an anti-tuberculosis drug that can cause hepatotoxicity in 20% of patients that is usually associated with an inflammatory response. Hepatocytes when exposed to non-toxic levels of H2O2, to simulate H2O2 formation by inflammatory cells, became twice as sensitive to isoniazid toxicity. Isoniazid cytotoxicity was prevented by 1-aminobenzotriazole, a non-selective P450 inhibitor or by bis-p-nitrophenyl phosphate (BNPP), an esterase inhibitor. Moreover, the cytotoxicity of hydrazine, the metabolite formed by amidase-catalyzed hydrolysis of isoniazid, was increased 16-fold by a non-toxic H2O2-generating system. The acetylhydrazine metabolite was found to be much less cytotoxic than hydrazine in this hepatocyte inflammation model. Hydrazine, therefore, seems to be the isoniazid reactive metabolite in this inflammation model. The molecular mechanism of hydrazine-induced cytotoxicity was attributed to oxidative stress as ROS and protein carbonyl formation occurred before the onset of hepatocyte toxicity. Hydrazine toxicity also involved significant production of endogenous H2O2 which resulted in lysosomal membrane damage which lead to a collapse in mitochondrial membrane potential. These results implicated H2O2, a cellular mediator of inflammation, as a potential risk factor for the manifestation of adverse drug reactions, particularly those caused by hydrazine containing drugs.

1211 CHOLOROACETONITRILE-INDUCED CYTOTOXICITY AND OXIDATIVE STRESS IN ISOLATED RAT HEPATOCYTES.

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Chloroacetonitrile (CAN) is a disinfectant by-product of chlorination of drinking water. The objective of the present work is to investigate the cytotoxic effects as well as the oxidative stress induced by CAN in isolated rat hepatocytes. Hepatocytes were isolated by a collagenase perfusion technique and were exposed to different concentrations of CAN (5–40 μM) in a time-course experiment for up to 2 h. CAN induced a significant decrease in cell viability and a significant increase in the leakage of hepatic enzymes. CAN exposure results in a significant decrease in cellular GSH content as well as a significant enhancement of TBARS accumulation in concentration and time-related manners. The obtained results suggested that 2h-exposure of hepatocytes to CAN (10 μM) was accompanied by submaximal responses. Therefore, a subsequent dose-response experiment was designed to evaluate the role of GSH modulation and oxidative stress in CAN toxicity in hepatocytes at 2 h. LDH release and TBARS generation were used as indicators in this experiment. Pretreatment with chlorodinitrobenzene (CDNB), buthionine sulfoximine (BSO), or bis(chloroethyl)-nitrosurea (BCNU) enhanced the cytotoxicity of CAN. Conversely, pretreatment with GSH or sulfhydryl compounds such as methionine (MT), cysteine (CYS) or dithiothreitol (DTT) attenuated CAN toxicity. Similarly, co-incubation with enzymatic antioxidants, superoxide dismutase (SOD) or catalase (CAT) or iron chelator, desferroxiamine (DFO) or the hydroxyl radical scavengers, mannitol (MAN) or dimethylsulfoxide (DMSO) exhibited significant protection against CAN cytotoxicity. In conclusion, our results suggest that CAN has a potential cytotoxic effect in isolated rat hepatocytes.

1212 4-HYDROXYNONENAL AND 4-OXONONENAL MAY CONTRIBUTE TO HEPATOSTEATOSIS BY DISRUPTING CELLULAR MICROTUBULES.

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Chronic ethanol consumption causes oxidative stress and lipid peroxidation in the liver, and is associated with the accumulation of lipid droplets in hepatocytes, a condition known as steatosis. The mechanisms by which steatosis occurs are currently under investigation. Intact microtubules are critical for the secretion of triglycerides from hepatocytes in the form of VLDL. We therefore investigated the hypothesis that disruption of the microtubule cytoskeleton in hepatocytes by reactive aldehydes could contribute to steatosis by reducing the secretion of triglycerides. Our recent experiments show that the lipid peroxidation products 4-hydroxynonenal (4-HNE) and 4-oxononenal (4-ONE) impair microtubule assembly, both with purified tubulin protein, and in the WIF-B hepatic cell line. Specific Cys residues adducted by 4-HNE were identified by tandem mass spectrometry and were found to occur in the region of interface between the alpha and beta tubulin subunits, suggesting a mechanistic basis for the impairment of tubulin function by 4-HNE and 4-ONE. Immunocytochemistry and confocal microscopy experiments showed that both 4-HNE and 4-ONE alter the polymerization state of tubulin in WIF-B cells with an overall decrease in intact microtubules. Tubulin extraction experiments likewise showed an increase in the ratio of soluble to polymerized tubulin after exposure of WIF-B cells to 4-HNE and 4-ONE. Additional experiments using Nile Red revealed that exposure of WIB-B cells to sub-lethal concentrations of 4-HNE and 4-ONE leads to impairment of neutral lipid secretion. These data demonstrate that covalent modification of proteins, including tubulin, by 4-HNE and 4-ONE can have long-lasting effects in the cell, and suggest that impairment of the microtubule transport pathways by the lipid aldehydes 4-HNE and 4-ONE may contribute to the accumulation of lipid in the alcoholic liver.

1213 MITOCHONDRIALLY-TARGETED DELIVERY OF A STABLE NITROXIDE PROTECTS HUMAN HEPATOCYTES FROM TROGLITAZONE- OR DICLOFENAC-INDUCED CELL INJURY.

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Troglitazone or diclofenac, drugs that have been associated with idiosyncratic liver injury in patients, both cause mitochondrial permeabilization and lethal cell injury in hepatocytes. However, the signaling pathways and the underlying mechanisms

are incompletely understood. We have previously demonstrated that both drugs increase the superoxide anion net levels specifically in mitochondria. If this mitochondrial oxidant stress was causally related to cell death, then mitochondrial targeting of a superoxide scavenger and antioxidant would not only prevent mitochondrial stress but would also protect from mitochondria-mediated cell injury. To test this hypothesis, we exposed the metabolism-competent immortalized human hepatocyte cell line (HC-04) to cytotoxic concentrations of troglitazone or diclofenac for up to 48 h in the presence or absence of mito-carboxy proxyl (mito-CP). Mito-CP is a stable nitroxide that accumulates in the inside negative mitochondrial matrix due to its cationic triphenylphosphonium moiety, acting as a radical scavenger and superoxide dismutase mimetic. We found that troglitazone (>75 μM) caused oxidation of mitochondrial thioredoxin-2 and activation of Ask1, which in turn initiated mitochondrial injury and cell death. In contrast, diclofenac (>500 μM) caused thioredoxin-2-independent activation of Bid, Bax and Bak, which triggered mitochondrial outer membrane permeabilization and cell death. However, in both cases, mito-CP (>10 nM) prevented the increase in superoxide and fully protected from cell injury. In conclusion, upstream inhibition of mitochondrial oxidant stress and signaling by the mitochondrially targeted mito-CP protected from more distal events that all lead to mitochondria-mediated cell death. These data are compatible with our hypothesis that troglitazone or diclofenac cause hepatocyte injury via mitochondria-selective oxidant stress and redox signaling.

1214 PROBING THE ROLE OF REACTIVE OXYGEN SPECIES ON ACQUISITION OF HEPATITIS B VIRUS HOTSPOT MUTATIONS IN VITRO.

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Background: Acquired mutations in host-integrated hepatitis B virus (HBV) DNA are common in human hepatocellular carcinoma (HCC) in Asia. Major changes include a double mutation in the basal core promoter (A1762T/G1764A) and in the pre-core region (G1896A). The presence of these mutations is associated with HCC development; unfortunately the mechanism of formation is unclear. HepG2 2.2.15 HCC cells stably transfected with the HBV genome were used to test the hypothesis that reactive oxygen species (ROS), often elevated in human disease progression towards HCC, induce HBV hotspot mutations.
Methods: HepG2 2.2.15 and HepG2 cells, a non-HBV transfected comparison, were treated with diquat to elevate intracellular ROS. Cell viability following treatment was determined by the MTT assay. Intracellular ROS levels in viable cells were assessed following diquat treatment by flow cytometric detection of CM-DCFDA fluorescence. Both cell and shed viral DNA in media were examined using a real-time PCR method for determining mutation acquisition.
Results: HepG2 2.2.15 cells are twice as sensitive to diquat toxicity compared to HepG2 cells. Measurements following diquat administration revealed that treated HepG2 2.2.15 cells also have over twice the level of intracellular ROS when compared to control cells. While none of the explored HBV hotspot mutations were detected in cell or viral DNA following 24 hour treatment with diquat, the endogenous production of this DNA damaging species portends mutagenic potential.
Conclusions: Experimental results show that a low concentration of diquat is effective in elevating intracellular levels of ROS in HepG2 2.2.15 cells. These cells also display increased sensitivity to diquat when compared with HepG2 cells; however, acute diquat treatment for 24 hours was not capable of generating HBV hotspot mutations. Long-term treatment experiments are now being conducted to determine whether continual cell passage in diquat generates these critical HBV hotspot mutations.

1215 APPLICATION OF STEATOSIS ASSAY FOR THE ASSESSMENT OF HUMAN HEPATOTOXICITY : ONO-NT-126, A THROMBOXANE RECEPTOR ANTAGONIST, INHIBITS MITOCHONDRIAL BETA-OXIDATION AND INDUCES STEATOSIS.

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Preclinical in vitro models that predict human liver toxicity will improve the safety profile of lead compounds prior to animal studies and increase the efficiency of drug discovery. Mitochondria are involved in fatty acid beta-oxidation, the tricarboxylic acid cycle, and oxidative phosphorylation, which provide most of the cell energy. It was therefore to be expected that mitochondrial dysfunction should be a major mechanism of drug-induced hepatotoxicity. We have constructed a steatosis assay for hepatotoxicity screening of compounds using Adipo Red staining in human hepatocytes, Fa2N-4 cells. Drugs (i.e. diclofenac, indomethacin, ibuprofen, tianeptin and perhexillin) which inhibit beta-oxidation in the mitochondria showed significant increase of Adipo Red staining in the Fa2N-4 cells, or induced steatosis. One hundred seven compounds which are known to induce hepatotoxicity in human were tested in this system, and the potencial of hepatotoxicity was detected with sensitivity of 34% and specificity of 88%. ONO-NT-126, a thromboxane receptor antagonist, which has a fatty acid side long chain in structure formula, is considered to be metabolized mainly by beta-oxidation and caused steatosis in this assay. In addition, a formation of beta-oxidation products during incubation of palmitic acid with hepatocyte was inhibited in the presence of ONO-NT-126. Furthermore, drug-induced steatosis was associated with lipid peroxidation as demonstrated by an increase in 8-isoprostane release. In conclusion, these data suggest that the steatosis screening should be informative and helpful for the prediction of liver toxicity, and that steatosis could induce oxidative stress such as lipid peroxidation which might lead to cell injury. ONO-NT-126 also might induce steato-hepatotoxicity in animals.

1216 3, 3', 4, 4', 5-PENTACHLOROBIPHENYL (PCB 126) DEREGULATES PROLIFERATION OF CONTACT-INHIBITED RAT LIVER EPITHELIAL 'STEM-LIKE' CELLS *VIA* ARYL HYDROCARBON RECEPTOR – EFFECTS ON CELL CYCLE REGULATORS, CELL-TO-CELL COMMUNICATION AND INTERACTIONS WITH OTHER SIGNALING PATHWAYS.

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A number of studies have suggested that aryl hydrocarbon receptor (AhR) ligands can disrupt cell cycle control leading to either inhibition or enhancement of cell proliferation in various cellular models. We have previously shown that, like the prototypical AhR ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), non-ortho and mono-ortho PCBs can disrupt contact inhibition and induce cell proliferation in rat liver epithelial WB-F344 cell line, in vitro model of liver oval cells. The mechanism of TCDD-induced disruption of cell proliferation control in this cellular model seems to involve overexpression of JunD transcription factor and upregulation of cyclin A, which overcomes G1 arrest associated with contact inhibition. In the present study, we show that, like TCDD, PCB 126 seems to disrupt cell cycle control in AhR-dependent and ARNT-independent manner. The effects of TCDD and PCB 126 are significantly modulated by pro-inflammatory cytokines, such as tumor necrosis factor-alpha and interleukin-6. These cytokines are extensively produced in diseased or regenerating liver, and we show that they can differentially modulate AhR signaling associated with deregulation of cell proliferation, suggesting that other signaling pathways may interact with AhR in regulation of cell proliferation. Both TCDD and PCB 126 induced significant changes in expression pattern of proteins associated with cell-to-cell communication, such as cadherins, catenins and connexins, and cell cycle regulation. Activation of AhR by its persistent ligands may thus lead to multiple changes in liver progenitor cells, possibly contributing to tumor promotion [Supported by the Czech Science Foundation - project No.524/06/0517].

1217 ROLE OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-α(IN OROTIC ACID-INDUCED HEPATIC STEATOSIS.

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Hepatic steatosis caused by drugs is an important component of the overall pathogenesis of drug-mediated liver injury. Dietary orotic acid (OA) is known to cause fatty liver in experimental animals. In general, fatty liver is caused by the impairment of fatty acid oxidation and the decrease in the secretion of apolipoprotein from the liver. We tested the effects of OA on the transcriptional activity of peroxisome proliferator-activated receptor-α(PPAR-α) and the expression of the target genes using PPRE-reporter gene assay and quantitative real-time PCR in H4IIEC3 cells, respectively. Target genes for PPAR-α include acyl-CoA oxidase (Acox), acyl-CoA dehydrogenase, short/branched chain (Acadsb), L-3-hydroxyacyl-CoA dehydrogenase, short chain (Hadhsc), 3-keto-acyl-CoA-thiolase (Thiol), and acyl-CoA dehydrogenase, very long chain (Acadvl). The changes in the expression of microsomal triglyceride transfer protein (MTP) which is responsible for the secretion of apolipoprotein from the liver was also tested. Treatment of H4IIEC3 cells with OA (20uM) repressed the transcriptional activity of PPAR-α. Accordingly, the expression of PPAR-α target genes, such as Acox, Acadsb, Hadhsc, and Acadvl was decreased significantly. We also found that MTP expression level was reduced by OA in a dose dependent manner. Long-term treatment of OA for 9days did not inhibit MTP expression. Taken all these data together, transsuppression of OA-induced

PPAR-α and, thus, the decreased expression of genes responsible for the β-oxidation of fatty acid and the lipid export from the liver, may contribute to the development of hepatic steatosis.

1218 SEX-RELATED DIFFERENCES IN METHIONINE TOXICITY IN FRESHLY ISOLATED MOUSE HEPATOCYTES AND EVIDENCE FOR TRANSAMINATION, BUT NOT TRANSMETHYLATION, PLAYING A KEY MECHANISTIC ROLE.

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L-methionine (Met) has previously been implicated in parenteral nutrition-associated cholestasis in infants and at high levels causes liver toxicity in animal models. Here, Met toxicity was characterized in freshly isolated male and female mouse hepatocytes incubated with or without Met for 0 to 5 h. In male hepatocytes, 20 mM Met was cytotoxic at 5 h while 30 mM Met was cytotoxic at 3 h as indicated by trypan blue and LDH viability assays. Apoptosis was not detected, suggesting cell death was necrotic. Interestingly, female hepatocytes were completely resistant to Met-induced cytotoxicity at these concentrations. The effects of aminooxyacetic acid (AOAA), an inhibitor of Met transamination, and 3-deazaadenosine (3-DA), an inhibitor of S-adenosylhomocysteine hydrolase, on Met toxicity in male hepatocytes were then examined. Reduced (GSH) and oxidized glutathione (GSSG) levels were also determined in cells and medium. Both 30 mM and 20 mM Met depleted GSH (at 2 and 3 h, respectively) without increasing GSSG levels. Addition of 200 μM AOAA protected against Met-induced cytotoxicity at 3 h (80 ± 19% and 91 ± 11% of control viability levels by trypan blue and LDH, respectively, compared to 55 ± 11% and 60 ± 13% for cells exposed to Met alone; means ± SD). GSH depletion was also reduced (59 ± 11% of control levels) compared to cells exposed to Met alone (24 ± 19% of control levels). Exposure to 0.3 mM 3-methylthiopropionic acid, a known Met transamination metabolite, caused GSH depletion and cytotoxicity. Addition of 100 μM 3-DA did not alleviate Met-induced cytotoxicity and GSH depletion. Collectively, these results suggest that Met transamination plays a major role in Met toxicity. Mouse hepatocyte sex-differences in Met toxicity may provide a useful model for further mechanistic studies. (supported by NIDDK44295 and T32-ES-007015).

1219 MECHANISMS FOR ENHANCED TNFα EXPRESSION BY WYETH 14, 643 IN RAT KUPFFER CELLS.

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Kupffer cells, resident macrophages in the liver, represent a major population (20%) of the hepatic nonparenchymal cells. Kupffer cells are found within the sinusoidal lumen, adhering to the liver sinusoidal endothelial cells, and are a rich source of growth regulatory factors in the liver, such as tumor necrosis factor α (TNFα). Kupffer cells have been reported to participate in the hepatocellular proliferation induced by Wyeth 14,643, a PPARα agonist, and well-established hepatocarcinogen in rodents. However, the mechanisms by which Wyeth 14,643 activates Kupffer cells are not clearly understood. The aim of the present study was to clarify the mechanisms leading to Kupffer cell activation following Wyeth exposure in vitro. To understand whether Wyeth directly affects Kupffer cells, we established a novel system for collecting highly purified primary rat Kupffer cells using in situ collagenase perfusion, elutriation and cell sorting (ED2+ cells), and for culturing the cells using macrophage colony-stimulating factor in vitro. Time course studies showed that cell cultures exhibited the mature phenotype and function of Kupffer cells after 7 days in culture. While LPS produced marked increases in protein and mRNA levels of TNFα and IL-6, Wyeth (12.5-100 uM) increased TNFα and IL-6 to a lesser extent. However, Wyeth (12.5-100 uM) significantly increased ROS production (upto 8.4-fold over control) in Kupffer cells. In addition, pretreatment with Wyeth appeared to sensitize Kupffer cells to LPS challenge, resulting in an enhanced (upto 2-fold increase) TNFα production. Furthermore, direct contact between Kupffer cells and hepatocytes enhanced Wyeth-induced TNFα release (> 2-fold increase). Wyeth 14,643 pretreatment also resulted in an increase in LPS-induced phosphorylation of the extracellular signal-regulated kinase (ERK) 1/2 and NF-kB activation in Kupffer cells. These results demonstrate that Wyeth may increase TNFα production in Kupffer cells through LPS-mediated signals. Supported in part by NIH CA100908.

1220 PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR α (PPARα) AGONISTS SUPPRESS ETHANOL-INDUCED OSTEOPONTIN EXPRESSION IN BILIARY EPITHELIAL CELLS.

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Recent studies from this laboratory have shown that osteopontin (OPN) mediates hepatic inflammation in a rodent model of alcoholic liver disease (ALD). Also, alcohol ingestion has been reported to inhibit hepatic peroxisome proliferator-activated receptor α (PPARα) activity leading to hepatic steatosis and inflammation. Therefore, the objective of this study was to investigate the potential relationship between the anti-inflammatory PPARα and proinflammatory OPN in cell cultures of hepatocytes and biliary epithelium. Experiments were designed to test the hypothesis that PPARα exerts anti-inflammatory effect by decreasing OPN expression. First, cells exposed to varying physiological and supraphysiological doses of ethanol (EtOH), lipopolysaccharide (LPS), a combination of EtOH and LPS, and acetaldehyde (ACA) resulted in increased OPN expression as indicated by Western blotting and mRNA expression by real-time PCR. ACA appeared to induce higher amount of OPN expression. Pretreatment of PPARα agonists (Wy14643; 50μM and bezafibrate; 150μM) resulted in significant reduction of OPN expression in EtOH, LPS, EtOH plus LPS, or ACA treated biliary epithelial cells. The mechanism by which PPARα agonists mediate decreased OPN expression appear to be due to inhibited nuclear transcription factor kB (NF-kB) binding to OPN promoter, which was shown by NF-kB inhibition experiment using NF-kB inhibitor, pyrrolidine dithiocabamate (PDTC). Studies are underway to explain the molecular basis by which PPARα inhibit OPN transcription through NF-kB. In conclusion, the current study suggests that OPN is induced both by ethanol and its metabolite acetaldehyde, and a negative relationship exists between PPARα and OPN expression.

Keywords: osteopontin, ethanol, peroxisome proliferator-activated receptor α

1221 EFFECT OF 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) ON THE EXPRESSION OF APOPTOSIS-RELATED GENES IN MOUSE LIVER AND PRIMARY RAT HEPATOCYTES.

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TCDD was classified as a group 1 carcinogen by the IARC though the mechanisms of TCDDs carcinogenic effects are to a great extent still unknown. TCDD acts as a strong tumor promoter in the liver of rats pretreated with a genotoxic carcinogen. This effect is believed to be caused by the clonal expansion of preneoplastic hepatocytes due to inhibition of apoptosis.

Microarray experiments analyzing genes involved in stress and toxicity (SuperarrayTM) were performed with hepatic RNA isolated from single-dosed, TCDD-treated wild type C57/BL6, and from transgenic mice, expressing a constitutively active AhR (CA-AhR).

Microarray data indicated differential expression of hepatic genes involved in apoptotic processes for both the TCDD-treated wildtype and the CA-AhR transgenic mice. In particular, mRNA levels of the anti-apoptotic gene Bcl-xL, but also of p21, cyclin D1, and caspase 1 were found to be induced in one or both types of animals. Enhanced expression of the Bcl-xL protein may lead to inhibition of apoptosis and result in survival of preneoplastic hepatocytes, ultimately giving rise to liver tumors. Additionally we investigated if TCDD also induced Bcl-xL in primary cultures of rat hepatocytes. Cells were incubated with various TCDD concentrations, dexamethasone served as a positive control. Following incubation with TCDD, a clear concentration- and time-dependent induction of CYP1A1 was observed. After >18 hours of incubation, dexamethasone showed an approximately two-fold induction of Bcl xL expression both in qRT-PCR and Western Blot analysis, whereas none of the TCDD concentrations used exhibited any effect on mRNA or protein levels of Bcl-xL. The differences between the microarray results and the in-vitro data may be due to interspecies-differences and suggest that Bcl-xL is not a common crucial factor in dioxin-induced tumor promotion in rodent liver.

1222 PRECLINICAL MECHANISTIC INVESTIGATION FOR RAT SPECIFIC HYPERBILIRUBINEMIA INDUCED BY MLN8054.

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Oral administration of MLN8054 to Sprague-Dawley rats at a dose of 125 mg/kg/d for 7 days resulted in a reversible icterus with hyperbilirubinemia (HB) characterized by an elevation in both bilirubin and bilirubin glucuronides. The HB was not observed in beagle dogs after high oral dose of MLN8054 at 2 mg/kg/d for 7 days. Similar concentrations of MLN8054 were observed in the rat and dog livers. However, the concentration of MLN8054-G (MLN8054-glucuronide, the major metabolite of MLN8054) was approximately 20 times higher in rat liver (≥ 6μM) than in dog liver. In the absence of any explanatory hepatic histological

changes alternative mechanisms for the HB were investigated. Bilirubin is taken up into hepatocytes by the sinusoidal membrane transporter, OATP, glucuroniated by UGT1A1 in the hepatocytes and then effluxed into bile through the bile canalicular membrane transporter, MRP2 and into blood via MRP3 mainly as bilirubin glucuronides. MRP2 and MRP3 expressing membrane vesicles, hepatocyte and Caco-2 cells were used to assess the inhibitory effect of MLN8054 and MLN8054-G on MRP2, MRP3, OATP and P-gp. Both MLN8054 and MLN8054-G inhibited P-gp-mediated taxol efflux in Caco-2 cells and inhibited MRP2 and MRP3 mediated LTC4 and E17βG uptake by MRP2 and MRP3 expressing membrane vesicles. MLN8054 and MLN8054-G also inhibited OATP-mediated E17βG uptake by rat hepatocytes. MLN8054 was a weak inhibitor of UGT1A1 in rat liver microsomes. The inhibitory effect of MLN8054-G on the bilirubin uptake transporter OATP and the bilirubin efflux transporters P-gp, MRP2 and MRP3, was greater than that of MLN8054, with the IC50 for MLN8054-G being ≤ 6 μM. Thus, the HB in rats may have been caused by the inhibition of transporters involved in the disposition of bilirubin by MLN8054 metabolite MLN8054-G. Since the formation of MLN8054-G in human liver microsomes was 10-fold lower than in rat liver microsomes and the projected human dose is low, the potential for MLN8054-induced HB in human is expected to be low.

1223 EFFECTS OF DILTIAZEM AND LANTHANUM ON COPPER INHIBITION OF MITOCHONDRIAL O_2 CONSUMPTION OF GILL OF THE AMERICAN OYSTER, *CRASSOSTREA VIRGINICA.*

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Previously, our lab demonstrated that tissues of the bivalve mollusc *Crassostrea virginica* could accumulate from their surroundings copper and other metals in excess of environmental levels. We also demonstrated that mitochondrial O_2 consumption in *C. virginica* gill and isolated gill mitochondria was inhibited by *in vitro* copper treatments. This is particularly interesting because many locations where *C. virginica* inhabit are copper polluted environments. In this study we determined the effects of two metal transport blockers, diltiazem and lanthanum, on the inhibitory actions of copper on O_2 consumption in gill mitochondria of *C. virginica.* O_2 utilization was measured using a YSI Micro-Biological Oxygen Monitor with a microbatch chamber. Addition of $CuSO_4$ (1-20 mM) to the chamber caused a dose dependent decrease in gill mitochondrial O_2 consumption. The metal blocker diltiazem (5-10 mM) did not alter mitochondrial O_2 utilization and its presence was able to significantly reduce by over 50% the inhibitory effect of copper treatments on respiration. The metal blocker lanthanum (5-10 mM) was less effective at protecting against the inhibitory effects of added copper, and in addition, contributed its own inhibitory effect on gill mitochondrial O_2 utilization. The study demonstrates that under our experimental condition, diltiazem is effective in blocking copper's inhibitory effects on gill mitochondrial O_2 consumption and may be useful in protecting against the effects of toxic copper uptakes in tissues of other animals as well. The toxic effects of copper on gill mitochondrial could be of physiological significance to the growth and long-term health of oysters and other marine animals living in a copper polluted environment and a copper uptake blocker could be of benefit for preventing diseases caused by high serum copper levels in humans. This work was supported in part by grants 2R25GM06003-05 of the Bridge Program of NIGMS, 0516041071 of NYSDOE, 0622197 of the DUE Program of NSF and 67876-0036 of PSC-CUNY.

1224 THE TOXIC EFFECTS OF MARINE BIOCIDE COPPER PYRITHIONE (COPPER 2-PYRIDINETHIOL-1-OXIDE) ON JUVENILE BROOK TROUT, SALVELINUS FONTINALIS.

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Copper pyrithione, a booster biocide developed to replace globally-banned tributyltin,acts by disrupting cell membranes, disrupting pH-gradients, and inhibiting ATP-synthesis in target species. However, research concerning the effects of CuPT exposure on non-target species is lacking. This investigation studies the acute effects of copper pyrithione exposure on juvenile brook trout, Salvelinus fontinalis. Morphologic changes, as well as copper bioaccumulation in several organ systems were studied. Brook trout were housed in a 950L aquarium and acclimated to laboratory conditions for 1 month. Trout were divided into groups of 10 and exposed to CuPT (dissolved in DMSO) in separate aquaria for a duration of 2 hours. Concentrations of CuPT ranged from 1ppm to 64ppm. Trout were sacrificed and dissected. Liver, brain and the 2nd and 3rd gill arches were removed and either frozen in liquid N2 or fixed for light or electron microscopy. Frozen tissue was digested in concentrated nitric acid and subjected to Inductively Coupled Plasma Atomic Absorption Spectroscopy (ICPAAS). Scanning electron microscopy was also performed on whole gill tissue. Results from histological analysis demonstrate the formation of club shaped lamella, significant epithelial lifting and edema, and the fusion of secondary lamella at CuPT concentrations of 16, 32 and 64ppm. AAS analysis demonstrates significantly increased levels of copper in the liver ($P < 0.001$) brain ($P < 0.005$) and gill arches ($P < 0.001$) of trout as compared to matched controls. Transmission electron microscopy revealed the presence of significantly altered mitochondrial morphology within chloride cells of gill tissue. They showed a swollen appearance with disruption of internal cristae. Scanning electron microscopy revealed significant epithelial exfoliation, fusion of adjacent lamella, and a loss of microridge structure. This preliminary data indicates that the use of CuPT as an alternate biocide could potentially harm non-target aquatic organisms.

1225 THE EFFECTS OF COPPER-DIMETHYLDITHIOCARBAMATE ON MITOCHONDRIAL-DEPENDENT APOPTOSIS AND HEAT SHOCK PROTEIN 60 IN RAT HIPPOCAMPAL ASTROCYTES.

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The decreased use of chromated copper arsenate (CCA) has led to the use of fungicidal alternatives such as copper-dimethyldithiocarbamate (CDDC) in pressure treated wood. Dithiocarbamates have been shown to induce reactive oxygen species (ROS), deplete antioxidant levels and induce oxidative damage. Stress upon cells caused by dithiocarbamate exposure may exhaust the capacity of heat shock proteins (HSPs) to protect cells, resulting in oxidized protein aggregation and misfolding. HSPs are considered to be a part of the antioxidant defense array. Chaperones are a class of HSPs that when overwhelmed result in disturbances in cellular metabolic processes causing the cell to undergo apoptosis. Heat Shock Protein 60 is localized primarily in the mitochondria in mammalian cells and little is known about its role in apoptosis. HSP60 aids in folding proteins in the mitochondria and in degradation of misfolded and denatured proteins. The relationship between mitochondrial-dependent apoptosis and the induction of mitochondrial HSPs is described. Rat hippocampal astrocytes were treated for 1 hour with CDDC and observed 24 hours post treatment. The LC50 was determined to be 1.8μg/mL. A decrease in cell number and morphological alterations including withdrawal of processes and distortion of cell shape were seen over a 24 hour period. Western Blot analysis of cytochrome c release from the mitochondria into the cytosol 12 hours post treatment was performed. Blots revealed that at 12 hours post treatment there was an increase of cytochrome c in the treated cytosol and a decrease in the treated mitochondria as compared to control. The TUNEL assay revealed that at 12 hours post treatment, numerous apoptotic bodies in CDDC treated cells were seen. Western Blot analysis of HSP60 expression indicated that there was a decrease but not a statistically significant difference between CDDC treated and control astrocytes.

1226 EFFECTS OF CADMIUM ON GROWTH AND METAMORPHOSIS OF AFRICAN CLAWED FROG.

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The objectives of this study were to determine the effects of Cd on tadpole growth, onset and completion of metamorphosis. African clawed frogs (Xenopus laevis) were exposed to 0, 1, 8, 85 and 860 μg Cd/L (measured concentrations) from fertilization to 86 days post fertilization. Tadpoles were collected randomly on days 31 and 49; and postmetamorphs (NF stage 66) were collected between days 49 and 86 as they completed metamorphosis. Endpoints measured included survival; snout vent length (SVL); tail length; total length; hindlimb length (HLL); weight; percent onset of metamorphosis (animals with forelimb emergence); and size and percentage of animals completing metamorphosis. Survival was not affected at any of the Cd concentrations tested until day 49 of exposure, but a significant decline in survival was observed in animals exposed to 860 μg Cd/L after day 49. No significant effects of Cd on tadpole growth parameters were observed at day 31. However, a significant reduction in SVL, HLL and total length were observed in samples exposed to 8 and 860 μg/L at day 49. Percent onset and completion of metamorphosis during the study period were not affected at Cd concentrations of 0 - 85 μg/L, but development was inhibited and metamorphosis was completely blocked at 860 μg/L. Size at metamorphosis was not affected by Cd at 1 and 8 μg/L but it was reduced at 85 μg/L. In conclusion, Cd at low concentrations inhibits tadpole growth in a bimodal manner whereas size at metamorphosis seems to be affected in a monotonic pattern. Thus, Cd at very low concentrations (8 μg/L) seems to inhibit tadpole growth rate but not its maximum size at metamorphosis, whereas the reverse situation occurs at higher concentrations (85 μg/L).

1227 BIOACCUMULATION OF MERCURY FROM MERCURY CONTAMINATED SOILS BY THE EARTHWORM LUMBRICUS TERRESTERIS.

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Earthworms can accumulate mercury (Hg) when grown in Hg contaminated soil (Earnst and Frey 2007). The purpose of this study was to determine if there was a correlation between the Hg concentration in soil and the amount of Hg accumulated in earthworms raised in the soil. Earthworms (Lumbricus terresteris) were grown in soils containing background, low and high concentrations of Hg. Worms from each exposure group were harvested at two week intervals and analyzed for total body Hg using cold vapor atomic absorption spectrophotometry. Worms grown in soil with the background concentration of Hg, 0.007 mg/kg, had a mean whole body concentration of 0.111 ug of Hg per g of freezed-dried tissue. Worms raised in soil with the low Hg concentration of 2.15 mg/kg had a mean tissue concentration of 219.03 ug/g. Worms grown in soil with the high Hg concentration of 4.37 mg/kg had a mean tissue concentration of 319.15 ug/g. Comparisons of Hg concentrations in the three groups with one-way ANOVA produced a P value <0.0001. Tukey-Kramer multiple comparisons test showed significant differences between the background and the low exposure groups (P<0.001), between the background and the high exposure groups (P<0.001), and between the low and the high exposure groups (P<0.001). Earthworms could provide a pathway whereby Hg contamination from atmospheric deposition enters a terrestrial food chain.

1228 TIERED TESTING APPROACH TO DETERMINE POTENTIAL ANDROGENIC RECEPTOR EFFECTS OF THE HUMAN PHARMACEUTICAL, INSPRA® IN FISH.

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Compounds that act on either the estrogen, androgen or thyroid receptors are considered endocrine active and therefore present a higher level of concern with regard to their potential impact to aquatic species. Inspra® (eplerenone) is an aldosterone antagonist indicated for congestive heart failure post myocardial infarction, as well as for the treatment of hypertension; and has been identified as a potential weak androgen receptor antagonist. To understand the potential for eplerenone to interact with the androgenic signaling system in fish, a tiered testing approach employing both an *in-vitro* radioligand binding assay with rainbow trout brain tissue and an *in-vivo* OECD 210 fathead minnow early life stage study were conducted. A radioligand binding study conducted with a rainbow trout brain cytosolic preparation showed that eplerenone was unable to displace [^{3}H] testosterone at all concentrations tested up to 100μM. In addition to eplerenone, testosterone, 11-ketotestosterone, flutamide and vinclozolin were assayed for their ability to bind to the androgen receptor. IC_{50} values for testosterone and 11-ketotestosterone were determined to be 3nM and 1155nM, respectively. Flutamide and vinclozolin did not bind to the testosterone receptor in this cytostolic preparation. These data along with the fathead minnow early life stage study demonstrated a low potential for Inspra® to act as an androgen receptor antagonist and were used as evidence to explain why a fish multigeneration study was not necessary.

1229 PROTEOMICS IN ECOTOXICOLOGY: PROTEIN EXPRESSION PROFILING TO SCREEN CHEMICALS FOR ANDROGENIC ACTIVITY.

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Protein expression changes can be used for detection of biomarkers that can be applied diagnostically to screen chemicals for endocrine modifying activity. In this study, Surface Enhanced Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (SELDI-TOF-MS) coupled with a short term fish assay was used to investigate changes in plasma protein expression as a means to screen chemicals for androgenic activity. Adult gravid female sheepshead minnows (*Cyprinodon variegatus*) were placed into separate aquaria for seawater control, ethanol solvent control, and the following androgen agonist treatments at 5.0 μg/L: dihydrotestosterone (DHT), methyldihydrotestosterone (MDHT), testosterone (T), methyltestosterone (MT) and trenbolone (TB). Treatments of 20, 40 and 80 μg/L chlorpyrifos (CP) served as non-androgenic negative controls. Test concentrations were maintained using an intermittent flow-through dosing apparatus supplying exposure water at 20 L/hour. Fish were sampled at 7 days, plasma diluted, processed on weak cation exchange CM10 arrays (BioRad) and analyzed. Plasma from treatments of the non-aromatizable androgen DHT was used to discover androgen-responsive plasma protein biomarkers. Multiple protein peaks, ranging from 2.9-55.3 KDa, were identified as putative markers of androgenic effects when comparing DHT treated and solvent control fish. Plasma from fish treated with the androgen agonists MDHT, MT and TB consistently exhibited expression of a subset of the DHT-responsive protein biomarkers. However, female fish from the non-androgenic CP treatments either failed to express or differentially expressed the androgen-specific biomarkers. The results indicate that changes in MOA specific diagnostic plasma proteins, as determined by SELDI-TOF-MS analysis, can be used to screen chemicals for androgenic activity.

1230 PROTEOMIC RESPONSES IN THE GILL AND MUSCLE OF ZEBRAFISH FOLLOWING IBUPROFEN EXPOSURE.

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The use of zebrafish as a model organism for drug discovery, target validation, and toxicological studies is rapidly growing in popularity and recognition. However, one of the key questions is the degree to which zebrafish respond in comparison to standardized mammalian models when treated with reference toxicants. To begin to understand the extent to which data from non-traditional organisms can be extrapolated across species, zebrafish were exposed to 10 ug/L of ibuprofen for ten days. Two-dimensional gel electrophoresis (2-DGE) revealed 39 up-regulated proteins, while 47 spots were down-regulated in the gill of ibuprofen exposed zebrafish. Two spots were significantly altered (i.e. 10 x differential) in zebrafish exposed to ibuprofen when compared to unexposed fish. LC-ESI-MS/MS was used to determine the identity of the significantly upregulated spots from gill and muscle. Preliminary identification suggests parvalbumin, and fatty-acid binding protein were significantly upregulated. Additional data will be presented profiling and identifying proteins with altered expression in zebrafish exposed to ibuprofen.

1231 PROTEOMIC AND GENE EXPRESSION CHANGES IN THE FATHEAD MINNOW LIVER IN RESPONSE TO FLUTAMIDE AND TRENBOLONE WATERBORNE EXPOSURE.

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Androgenic chemicals are present in the environment but have not received the attention given to pharmaceutical estrogens. In this study we determined whether a model anti-androgen (flutamide) and androgen (17-β trenbolone) affected the liver proteome after a 48 hour waterborne treatment. Flutamide is a pharmaceutical that is most commonly used in the treatment of prostate cancer and is a direct competitor for the androgen receptor. In contrast, trenbolone is largely used as a growth promoter for cattle. Female fathead minnows were exposed to a single dose of either flutamide (5, 50, or 500 μg/L), trenbolone (0.05, 0.5, or 5 μg/L), or a mix of 500 μg/L flutamide and 0.5 μg/L 17-β trenbolone for 48 hours. We used the iTRAQ® method (Applied Biosystems) to label peptides from a control, flutamide (500 μg/L), 17-β trenbolone (5 μg/L), and the mixed group for LC MS/MS analysis. In general, the mix of both chemicals showed an intermediate response in relative peptide abundance. Approximately 21 proteins had multiple peptides identified as differentially regulated by treatment (p<0.05). Candidate proteins regulated were involved in chromatin packaging (histone 1), translation (ribosomal S28, L23a, elongation factor-1), oxidative and general stress response, (catalase, HSP70), and cellular metabolism and signaling (betaine homocysteine methyl-transferase, calmodulin). Altered protein abundance correlated with mRNA levels in some cases (e.g. ribosomal L23a, catalase) but not others (e.g. betaine homocysteine methyltransferase), suggesting that effects were evoked at both transcription and translation. We demonstrate the utility of using both genomic and proteomic tools in identifying candidate biomarkers of chemical exposure.

1232 HEPATIC GENE EXPRESSION IN RAINBOW TROUT (ONCORHYNCHUS MYKISS) EXPOSED TO DIFFERENT HYDROCARBON MIXTURES.

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Traditional biomarkers for hydrocarbon exposure, such as EROD levels or CYP 1A, are not induced by all PAHs or by all petroleum substances. The objective of this study was to determine if exposure to a crude oil and different refined oils generated a hydrocarbon-specific response in gene expression profiles, and whether these gene expression profiles could be used as a generic biomarker for hydrocarbon exposure.

Juvenile rainbow trout (Oncorhynchus mykiss) were exposed via the water accommodated fraction (WAF) to sublethal concentrations of either kerosene, gas oil, heavy fuel oil or crude oil hydrocarbons for 96 hours in a novel, no headspace system. Tissue was collected for RNA extraction and microarray analysis using salmonid cDNA arrays (GRASP, University of Victoria). Genes that were significantly over or underexpressed were determined using a t-test, $p < 0.05$, multiple test correction. Exposure to each WAF resulted in different list of up and down regulated genes suggesting substance-specific hepatic responses. Exposure to crude oil WAF resulted in changes in expression of genes including CYP 1A and GST with known roles in detoxification pathways. Gene expression profiles generated in this experiment were compared to those from previous experiments, in which rainbow trout were compared to a diverse suite of environmental toxicants. Transcriptomic profiles resulting from the WAF exposures did not have many of the same genes significantly altered. In subsequent analyses, when data from this experiment was included in cluster analysis with data collected in previous experiments, the hydrocarbon exposures were grouped in some instances, depending on the gene list used. These initial results suggest that data mining techniques may result in groupings not immediately evident. These preliminary analyses suggest that multivariate transcriptomic-based techniques may be more appropriate than single gene studies in developing potential biomarkers of hydrocarbon exposure.

1233 SPECIFIC AHR AND ERALPHA IN VITRO TOXICITY OF CRUDE AND REFINED PETROLEUM PRODUCTS.

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Current legislation and risk assessment of petroleum products (oil) take account of nonspecific toxicity (narcosis) only. However, some researchers have observed specific toxicities for oils by using *in vitro* assays. Unfortunately, the information on such toxicity of oils remains fragmented. Most studies use a single assay or oil product. In the present study, we systematically investigated the specific toxicity of oils by exposing a large series of oil products to several different *in vitro* assays including H4IIE-DR-luc (Ah-receptor mediated toxicity), U2OS-ERa-luc (estrogen receptor alpha mediated toxicity), and the T-Screen (thyroid receptor mediated toxicity). The assays were dosed with 4 different crude oils, 1 lubricant and 5 fuel oils, covering very light to very heavy products. The dosing was performed at different concentrations of either pure oil solutions in ethanol or oil solutions in combination with the assay's positive control (BaP, TCDD, E2, T3, all at EC50 levels). In the H4IIE-DR-luc, most of the oils produced large responses after 6h of incubation. However, after 24h, only the heavy oils and one light refined product continued to have high responses. In the U2OS-ERa-luc assay, the highest responses were observed for fuel oils (one light and two heavy bunker oils), while in the T-screen none of oils affected cell proliferation. In addition, at all oil concentrations and in all assays, cell responses were high when combined with the assays' positive controls at EC50 levels. To conclude, our results indicate that crude oils and refined products may contain a suite of specifically acting toxicants, both persistent and easily metabolized compounds, whose effects can be significantly intensified when combined with other specifically acting chemicals.

1234 EFFECT OF *MICROCYSTIS AERUGINOSA* LIPOPOLYSACCHARIDE (LPS) ON NEONATAL RAT BRAIN MICROGLIA RELEASE OF SUPEROXIDE ANION, THROMBOXANE B_2, TUMOR NECROSIS FACTOR-α AND MATRIX METALLOPROTEINASE-9.

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Cyanobacteria ("blue-green algae") produce lipopolysaccharides (LPSs) similar to "endotoxins," the causative agent of septicemia typically associated with infection by various heterotrophic bacteria. In contrast to the well-studied endotoxins from heterotrophic bacteria, rather limited information exists on the toxicology of cyanobacterial LPSs. We investigated the LPSs from the freshwater cyanobacterial species,
Microcystis aeruginosa, which contaminates drinking water sources and recreational waterways worldwide. Specifically, we tested *M. aeruginosa* LPS (MaLPS) in rat brain microglia (BMG) as an *in vitro* model system, using release of tumor necrosis factor a (TNF-α), thromboxane B2 (TXB_2), superoxide anion (O_2^-) and matrix metalloproteinase-9 (MMP-9) as indicators of a neuroinflammatory response. MaLPS was isolated from *M. aeruginosa* strain UTCC 299 by hot phenol/water extraction. BMG were isolated from neonatal rats, and treated *in vitro* with MaLPS for 17 hours. TNF-α and TXB_2 were determined by immunoassay, O_2^- by cytochrome C reduction, lactate dehydrogenase (LDH) release by enzyme activity and MMP-9 by zymography. LDH release was observed for BMG treated at concentrations of MaLPS above 10 ng/mL. MaLPS, likewise, stimulated TNF-α and O_2^- release at 10 ng/mL, while TXB_2 and MMP-9 release above 100 ng/mL. Our current results appear to suggest that exposure to cyanobacterial LPSs may pose a potential threat to human and environmental health, and that further investigation of their chemistry and toxicology, particularly at the functional and molecular level, are needed. Supported by Abbott Laboratories, Midwestern University and Florida International University.

1235 EFFECTS OF BENZO[*A*]PYRENE ON MITOCHONDRIAL DNA DAMAGE IN THE KILLIFISH (*FUNDULUS HETEROCLITUS*).

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Previous studies have shown that mitochondrial DNA (mtDNA) is more susceptible to certain types of chemical damage and oxidative stress than nuclear DNA (nDNA). Therefore, a number of studies have assessed mtDNA damage in traditional laboratory animals such as mice and rats. However, few studies have addressed contaminant-induced mtDNA damage in aquatic model nor compared sensitivities in these models. Here, we adapted a long-range, quantitative polymerase chain reaction (QPCR) assay to measure both mtDNA and nDNA damage in killifish (*Fundulus heteroclitus*), a well-established environmental model. In this study, total DNA from selected tissues of fish (brain, liver, muscle) was isolated, and, specific regions of mitochondrial and nuclear DNA were amplified. Amplification was normalized by using PCR products of short length targets; lesion frequency was calculated to assess relative DNA damage. In fish treated with 10 mg/kg benzo[*a*]pyrene (BaP), all three tissues exhibited increased DNA damage in response to BaP exposure, and up to four-fold more damage in the mtDNA than nDNA. This difference, while significant, is much less than the differences between mtDNA and nDNA previously reported in mammalian models. Comparisons of fish from a PAH-polluted and reference sites show that fish from the polluted site have higher basal mtDNA and nDNA damage, but there was no significant increase in damage in response to subsequent BaP exposure. Results indicated that mtDNA is more sensitive to BaP treatment than nDNA in killifish. In addition, killifish from a PAH-polluted site appeared relatively resistant to DNA damage in response BaP exposure. This research was supported by the NIEHS-supported Duke University Superfund Basic Research Center (P42 ES10356), and Integrated Toxicology Program (T32 ES07031).

1236 EFFECTS OF POLYBROMINATED DIPHENYLETHER (DE-71) EXPOSURE ON STEROIDOGENSIS IN JUVENILE XENOPUS (SILURANA) TROPICALIS.

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The impact of the brominated flame retardant mixture, DE-71, on gonadal steroidogenesis during sexual differentiation in Xenopus tropicalis was examined because of its ubiquity in the environment, bioaccumulative nature, and potential to disturb endocrine activity. Exposure of Xenopus tropicalis to DE-71 during development was previously found to increase in the proportion of phenotypic females. A concentration-related increase in liver necrosis and abnormal ovary development were also observed. To further examine the effect of DE-71 exposure on gonadal differentiation, aromatase, 5α-reductase, and CYP17 activities were measured, in addition to; plasma estradiol (E2), testosterone (T), and dihydrotestosterone (DHT) levels. Gonad and plasma samples were collected from juvenile control specimens and organisms exposed to DE-71 from early embryo through metamorphosis. Decreased mean plasma DHT and T, and increased mean E2 levels were found in PFOS-treated juvenile X. tropicalis compared to controls. Mean gonadal aromatase activity in X. tropicalis exposed to DE-71 was not appreciably affected, but decreased mean 5α-reductase and CYP17 activities were observed compared to control frogs. Additional confirmatory studies with cultured undifferentiated X. tropicalis gonad explants suggested that PFOS was capable of decreasing 5α-reductase and CYP17, but did not alter aromatase activity in vitro. These results suggest that the decreased gonadal 5α-reductase and CYP17 activities combined with relatively consistent aromatase activity may have resulted in decreased plasma androgen and increased estrogen levels found in DE-71-treated X. tropicalis. Further, the increase in the proportion of phenotypic female frogs may be the result of altered steroidogenesis resulting from DE-71 exposure.

1237 MONITORING OF ENVIRONMENTAL POLYCYCLIC AROMATIC HYDROCARBONS (PAH) IN FISH, VEGETATION AND SOIL FROM THE CUCHARILLAS MARSHLAND, CATANO, PUERTO RICO.

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Polycyclic aromatic hydrocarbons (PAHs) are by-products from incomplete combustion of carbonaceous materials. PAHs have the capacity to move through the environment via the atmosphere, water and soil. Some of these PAHs are known to have carcinogenic effects in humans and animals. Plants that grow on PAH-contaminated soils have the capacity to absorb the compounds and bioaccumulate them. Soils are important reservoirs for the PAHs, and fish that inhabit contaminated areas can also bioaccumulate PAHs. Through the alimentary canal, humans can be exposed to these environmental carcinogens. The fact that the Cucharillas marshland is used for recreational activities presents a potential source of human exposure to these carcinogens. The aim of this study is to evaluate the presence of PAHs such as benzo[a]pyrene, benz[a]anthracene, and benzo[b]fluoranthene from Tilapia, soil and plants from the Cucharillas marshland. Tilapia, soils and local flora were obtained from contaminated areas in the marshland. For extraction and identification of PAHs from fish tissues, a microwave extraction technique was optimized and applied. As controls, Tilapias from Plata Lake were collected and analyzed. Identification of the monitored PAHs was made via HPLC-MS analysis. Plants were extracted by the Soxhlet technique and extracts were analyzed by HPLC-ESI-MS. For PAH extraction from soil, a sonication method is being applied to compare with the more traditional Soxhlet method. Co-chromatography gave preliminary results that indicate the presence of PAHs in fish at the μM level. In plants and soil, fewer PAHs have been identified. PAHs identified in fish, soils and flora were benzo[a]pyrene and benz[a]antrhracene. This study presents evidence of the presence of environmental carcinogens on the Cucharillas marshland.

1238 ASSESSMENT OF PERFLUORINATED CHEMICALS (PFCS) DEVELOPMENTAL TOXICITY USING EMBRYONIC ZEBRAFISH.

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Perfluorinated chemicals (PFC) have been used since the 1950s in many industrial and commercial applications because of their unique properties such as chemical inertness, resistance to heat, and their ability to repel water and oils. Concerns regarding potential environmental or human health risk from PFCs exposures have emerged as these chemicals are persistent in the environment and bioaccumulate in animal tissues. Early life stages are often sensitive to chemical insult, so it is essential to determine if PFCs are developmentally toxic. Forty-one structurally diverse PFCs, including perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), were screened for potential developmental toxicity using an embryonic zebrafish bioassay. In initial studies, developmental responses to waterborne exposure to PFCs were determined using a range of concentrations (0.01-200 μg/L), including levels that are relevant to the environment. The majority of the PFCs tested did not elicit any adverse developmental responses. However, increased embryonic malformations were observed in animals exposed to 17 PFCs. Since the embryonic dose cannot be inferred from waterborne exposures, six additional PFCs that are most commonly studied were also tested by microinjection into developing embryos with doses between 0.04-7.32 mg/kg. Three of these six PFCs elicited embryonic malformations, including mortality and head defects. These results show that PFCs are not teratogenic in general. However, exposure to three carboxylated PFCs, perfluorohexanoic acid (PFHxA), PFOA, and perfluorodecanoic acid (PFDA), at environmentally relevant levels did lead to embryonic malformations.

1239 THE DISPOSITION AND REPRODUCTIVE TOXICITY OF PBDE-47 AND PERFLUOROCTANOIC ACID IN FISH AFTER ORAL DOSING.

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The structurally dissimilar classes of environmental pollutants polybrominated diphenyl ethers (PBDEs) and perfluorinated alkyl compounds have become ubiquitous aquatic contaminants. Consumption of fish can be an important exposure route for these contaminants in both humans and marine mammals. This is worrisome due to potential effects on the thyroid system among other toxicological endpoints. In this study, we characterized the disposition of PBDE-47 and a perfluorinated compound, perfluorootanoic acid (PFOA) in rainbow trout (O. mykiss) and fathead minnows (P. promelas) after single and multiple oral doses. Additional experiments with fathead minnows studied the effects on reproduction and maternal transfer during 21 days of continuous oral dosing. After intra-vascular injection in trout, PBDE-47 was slowly excreted with an elimination half-life of 156 hours. After oral dosing, peak plasma levels were not attained until 9 hrs after dosing. This relatively slow absorption of PBDE-47 is reflected in the low absorption rate (ka) of 0.13 hr-1. Although slowly absorbed, estimation of systemic bioavailability was greater than 60 % for most trout. In fathead minnows, both PBDE-47 and PFOA were rapidly absorbed after oral dosing. There was evidence for sex differences in the accumulation of PFOA in minnows but not trout with male minnows having a higher body burden than female minnows. Repeated oral exposure to PBDE-47 decreased circulating thyroxin (T4) levels and reproductive output in actively breeding fish. Combined, these results suggest PBDEs and PFOA readily bioaccumulate in fish after oral exposure with important gender differences in toxicity and/or retention in fish.

1240 COMPARISON OF PREDICTED AND EXPERIMENTALLY DERIVED VALUES FOR THE DURATION OF THE EXPOSURE PHASE IN FISH BIOCONCENTRATION STUDIES.

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Regulatory guidelines for the conduct of fish bioconcentration studies allow for the duration of the exposure phase required to reach steady state concentrations of the test substance in fish to be predicted. The expected duration of the exposure phase can be estimated from the octanol/water partition coefficient (Pow) of the test substance and assumes that the uptake and depuration phases follow first-order kinetics. Using historical data, values for the duration of the exposure phase required to reach steady state were derived experimentally for fifteen radiolabelled compounds having log10 Pow values in the range of 2.8 - 9.2. The experimentally derived values for the duration of the exposure phase required to achieve steady state concentrations were in the range of the predicted values for only four out of the fifteen compounds. For four other compounds, the experimentally derived values were more than twice the predicted values. For one compound, the experimentally derived value was 1-2 times the predicted value and for the remaining six compounds, the experimentally derived values were less than the predicted values.
The regulatory trigger for the conduct of a fish bioconcentration study is a log10 Pow value > 3. The historical data for the 15 compounds showed that there was a poor correlation between the log10 Pow values and the measured steady state bioconcentration factors (BCFss). In most cases, bioaccumulation was lower than expected.
In conclusion when considering the design of fish bioconcentration studies, first-order kinetics cannot be assumed and metabolism of the test substance may be significant. Therefore sufficient test substance should be made available for possible extension of the exposure phase. The universally used octanol/water partition coefficient for a compound is a poor predictor for bioconcentration, although in most cases bioconcentration is less than expected due to metabolism of the compound by the fish.

1241 COMPARING THE TOXICITY AND BIOACCUMULATION POTENTIAL OF BULK AND NANO ALUMINUM OXIDE USING *EISENIA FETIDA.*

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Nano aluminum offers great promise in commercial and military technology applications such as coatings and thermites. Very few studies have been conducted to determine the effects of nano particles on terrestrial organisms such as the redworm, *Eisenia fetida*. The acute and chronic toxicity of bulk (50-200μm) and nano (11 nm) Al_2O_3 was assessed using *E. fetida*. Acute contact toxicity exposures were conducted for 72 hours using glass scintillation vials lined with Whatman #1, 9 cm circular filter paper. Solutions of nano Al_2O_3 in deionized water were prepared and distributed into vials in concentrations of 0.1, 0.3, 1, 3, and 10 mg/ml, plus a control. One organism was placed in each vial. At test termination, no mortality was observed. A chronic toxicity and bioaccumulation study was conducted to compare the effects of bulk versus nano Al_2O_3 in spiked reference soil. The soils were spiked in treatments of 0, 100, 300, 1000, 3000, 10000 mg/kg for both nano and bulk Al_2O_3 with an additional treatment of 13,000 mg/kg treatment for bulk Al_2O_3. At termination of both bioaccumulation studies, no significant mortality was found in either acute or chronic testing. Weight loss was observed in both acute and chronic experiments but did not appear to be treatment related. A significant decline in reproduction (as determined by the total number of cocoons recovered) was observed at the 10000 mg/kg concentration of nano Al_2O_3as compared to the control

(p=0.007) and bulk Al_2O_3 treatments at the same concentration. Bioaccumulation data will further contribute to our understanding of the effects data. From our preliminary results nano Al_2O_3 exposure did result in a significant reduction in *E. fetida* reproduction compared to bulk aluminum, although at relatively high levels in soil.

1242 DIALLYL SULFIDE INCREASES DIETHYLSTILBESTROL EXPOSED MCF10A CELL VIABILITY AND REDUCES DNA SINGLE STRAND BREAKS.

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Breast Cancer is one of the leading causes of death in American women. In 2003 181,646 women and 1,826 men were diagnosed with breast cancer of which 41,619 women and 379 men died. In a human embryonic cell viability experiment, cell death was seen with diethylstilbestrol (DES) at 20 µM. Diallyl Sulfide(DAS) has been shown to inhibit Diethylstilbestrol induced DNA adducts by 96% in the breast tissue of ACI rats. We hypothesize that DAS will prevent DES induced cell death and DNA strand breaks in MCF10A cells. To test this hypothesis we dosed MCF10A cells with DES (100uM) and various concentrations of DAS (1 uM and 10 uM) for 24 hours. Cell viability was determined via the MTS assay and DNA strand breaks were measured by Comet Assay Analysis using the Mean Olive Tail Moment as the parameter for measuring DNA strand breaks. We found that DES significantly increased the number of DNA strand breaks in a dose dependent manner. Furthermore DAS (1uM and 10uM) inhibited DES (100uM) induced DNA strand breaks by 34% and 53% respectively. DES reduced cell viability by 51% relative to the control. DAS (1uM and 10uM) augmented this reduction in cell viability by 10% and 33% respectively. Our data demonstrates the DAS inhibits DNA strand breaks and cytotoxicity induced by DES in MCF10A cells. The protective mechanisms afforded by DAS may play a role in the chemoprevention of estrogen induced breast cancer.

1243 EFFECT OF THE CYP1A INHIBITOR FLUORANTHENE ON THE BIOTRANSFORMATION OF BENZO[A]PYRENE IN TWO POPULATIONS OF *FUNDULUS HETEROCLITUS* WITH DIFFERENT EXPOSURE HISTORIES.

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Creosote-contaminated sediment from a Superfund site on the Elizabeth River (ER), VA is teratogenic to killifish (*Fundulus heteroclitus*) from reference sites. However killifish from the site are resistant to this teratogenicity. Mechanisms underlying the resistance are not well understood; however, ER killifish are refractory to induction of CYP1A mRNA, protein and activity. Fluoranthene (FL), a CYP1A inhibitor, and benzo[a]pyrene (BaP), an AHR agonist, are dominant polycyclic aromatic hydrocarbons (PAHs) at the site, and co-exposure causes embryotoxicity in reference site embryos. The purpose of this work is to determine how CYP1A inhibition affects the metabolism of BaP and ultimately, teratogenicity in killifish. We also examined how the ER CYP1A phenotype affects biotransformation of BaP and teratogenicity with and without FL, compared to the reference site (King's Creek, VA). Embryos were dosed with 10-400 ppb BaP with or without 500 ppb FL for 5 days. Induction of CYP1A, 1B1, and 1C1 mRNA, CYP1A enzymatic activity, and BaP metabolic profile were analyzed at 5 dpf, and cardiac deformities at 7 dpf. KC embryos showed significant induction of CYP1A, 1B1, and 1C1 mRNA, and CYP1A protein activity at all BaP concentrations. Co-exposure to FL did not alter mRNA induction, but decreased CYP1A activity and increased cardiac deformities. ER embryos showed no change in CYP1A activity or cardiac deformities for any treatment. Greater concentrations of BaP were detected in ER embryos exposed to 200 ppb BaP than KC embryos exposed to the same concentration; co-exposure with FL did not significantly alter BaP metabolism. We conclude that co-exposure to BaP and FL causes synergistic teratogencity in the KC, but not in the ER population. Killifish from the ER metabolize BaP at a slower rate than KC embryos, and the presence of FL has no significant affect on the rate of BaP metabolism. NIEHS-supported Duke University Superfund Basic Research Center (P42- ES-10356), and ITEHP (T32- ES-007031).

1244 INVESTIGATION OF THE LACK OF CYTOCHROME P450 1A1 GENE EXPRESSION IN PADDLEFISH (POLYODON SPATHULA).

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Paddlefish are a long-lived primitive fish species with a cartilaginous skeleton, inhabiting the Mississippi River drainage. Previous work in our laboratory indicated that PCB-contaminated paddlefish collected from the Ohio River did not have measurable hepatic microsomal EROD activity. In addition, we were unable to detect CYP1A1 protein in these paddlefish in a western blotting analysis using rabbit anti-trout CYP1A1 (LM4b) IgG antibody. The objective of this study was to investigate the lack of CYP1A1 expression in paddlefish. Using primers derived from the first and last exons of rainbow trout CYP1A1 gene, the polymerase chain reaction was used to amplify trout, sturgeon and paddlefish genomic DNA. PCR products of the correct size were generated for each species. Our results indicate that the paddlefish genome contains the CYP1A1 gene, but the previous evidence would suggest that it is not being expressed. Currently we are trying to detect the presence of the Ah-receptor necessary for promoting CYP1A1 production.

1245 REPRODUCTIVE TOXICITY OF NORETHINDRONE TO JAPANESE MEDAKA.

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Active pharmaceutical ingredients (API) and metabolites are routinely being detected in the environment, primarily due to post-consumer use. Most of the research focus has centered on the presence and toxicity of estrogenic and androgenic compounds. However, little data are available describing the impact of synthetic progestins on fish models. Due to this lack of data, sexually mature Japanese medaka were exposed to the model progestin, norethindrone, to evaluate reproductive responses and potential toxicity. The acute 48hr LC50 for norethindrone is > 1 mg/L, while the 7d NOEC and LOEC for growth is 250 and 500 ug/L, respectively. Reproductive studies suggest that this model progestin reduces fecundity at the ng/L level. Data will be presented focusing on reproductive responses of Norethindrone and if the reproductive responses are gender specific.

1246 CHARACTERIZATION OF METABOLIC ENZYMES AND METABOLIC STABILITY IN RAINBOW TROUT CRYOPRESERVED HEPATOCYTES AND S9 LIVER FRACTIONS.

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Bioaccumulation of xenobiotics (e.g. environmental chemicals) and their metabolites in organisms is of increasing interest to environmental protection agencies. Rainbow trout cryopreserved hepatocytes or S9 liver fractions are potential in vitro model systems that may be used to predict bioaccumulation potentials of chemicals by determining metabolic stability. The aim of this study is to compare activities for Phase I and II drug metabolizing enzymes in rainbow trout cryopreserved hepatocytes and liver S9 fractions. In addition, metabolic stability of two commercial chemicals with potential environmental impact, 17-β-Estradiol and diclofenac, were assessed to compare and to demonstrate feasibility of these model systems.

Cryopreserved hepatocytes were prepared from Rainbow trout sampled from a state hatchery. Fish were anesthetized with MS-222 and sacrificed. Livers were flushed with HBSS pH 7.8 at 11-13 oC through the hepatic vein. Hepatocytes were isolated by collagenase perfusion and cryopreserved in liquid nitrogen. Trout S9 fractions were prepared from flash frozen livers.

Phase I drug metabolizing enzymes in cryopreserved rainbow trout hepatocytes and liver S9 fractions were characterized for EROD (ethoxyresorufin deethylase) and testosterone hydroxylation activities. Phase II drug metabolizing enzyme activities were also measured for sulfotransferase (ST) and UDP-glucuronysyltransferase (UGT) activity using ethoxycoumarin. Incubations were performed at pH 7.8 and 10 °C to reflect physiological trout conditions. 17-β-Estradiol and diclofenac were incubated for 5 time points and disappearance of parent was measured by LC-MS/MS.

These results demonstrate the feasibility of Rainbow trout cryopreserved hepatocytes or S9 liver fractions for use as in vitro model systems to predict bioaccumulation of environmental chemicals.

1247 TOXICOGENOMIC RESPONSES OF RAINBOW TROUT EXPOSED TO CONTAMINANTS.

S. E. Hook and I. R. Schultz. *Battelle PND, Sequim, WA.*

A growing list of contaminants have been identified to negatively impact reproduction in fish. As part of our efforts to better understand the mechanistic basis for these effects, we have used DNA microarray technology to characterize gene expression patterns in rainbow trout (Onorhyncus mykiss) associated with sublethal, short-term exposure to different classes of toxicants including ethynylestradiol (xeno-estrogen), trenbolone (a model androgen), 2,2,4,4-tetra-

bromodiphenyl ether (BDE-47, thyroid active), diquat (oxidant stressor), chromium VI, and benzo[a]pyrene. In several studies, we have pursued questions associated with dose-response, time dependent changes in tissue specific gene expression and contaminant mixtures. These studies all used clonal hybrid rainbow trout, which reduces phenotypic variation and facilitates comparison of findings across different studies. Among the different classes of toxicants studied, the most persistent and identifiable gene expression signature is that obtained with xeno-estrogens. A meta-analysis of all toxicogenomic data collected by our laboratory demonstrated that the toxicogenomic response to xeno-estrogens can be distinguished from other contaminants regardless of dose, sampling time or age of fish. When other contaminants are present at toxicologically significant levels the gene expression pattern cluster separately. Thus, using toxicogenomic analysis it is possible to differentiate toxic responses that are primarily an estrogenic mode of action from those that are non-estrogenic or the result of mixed (multiple) modes of action. A similar type of analysis can be performed for the other modes of action (e.g. oxidative stress, androgenic, thyroid active) to expand the diagnostic capacity of toxicogenomic analysis in fish.

1248 SIMULTANEOUS ANALYSIS OF DOMOIC ACID KINETICS AND ELISA ASSAYS TO GUIDE PUBLIC HEALTH DECISIONS FOR MULTIPLE SHELLFISH SPECIES.

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Blooms of the marine algae Pseudo-nitzschia produce domoic acid (DA) which can cause amnesic shellfish poisoning in humans. Uncertainties in DA toxin uptake and depuration in shellfish limit our ability to infer which shellfish species have DA concentrations low enough to allow seafood consumption. Decision making can be improved by following a sequence of steps including Enzyme-Linked ImmunoSorbent Assay (ELISA) analysis to determine DA tissue concentrations, using these to infer DA ingestion and depuration rates, and using these rates to guide public health decisions. To reduce the errors from each step, we simultaneously model shellfish DA kinetics, the resulting ELISA outputs, ELISA calibration, and public health decisions. An ELISA plate with only one sample having a coefficient of variation <20% is used to demonstrate that cockles, blue mussels, and Pacific oysters allow rapid DA depuration (>1%/hour), while horse clams, littleneck clams, and cockles demonstrate ingestion rates slow enough to allow less frequent sampling for food safety. This simultaneous integrated approach allows rigorous decision making from limited experimental data.
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1249 BIOMARKERS OF EXPOSURE AND TOXICITY IN ECOLOGICAL RECEPTORS.

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Contaminated sediments represent a potential threat to human and ecological health. Due to the complexity of these materials, there is a great deal of uncertainty associated with risk characterization of sediments. A series of in situ studies were conducted at a Superfund site in the Pacific Northwest. River sediment at the site is contaminated with polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs). These studies are aimed at measuring biomarkers in caged juvenile Chinook salmon and Pacific staghorn sculpin. Based on existing data, four stations were selected for water and sediment sampling. Fish were caged and exposed at each of these stations for 7-8 days. Following sacrifice, fish tissues were analyzed for body burden of PAHs and PCBs, excluding gills and livers, which were dissected for DNA adduct analysis using ^{32}P-postlabeling. Levels of contaminants present in sediment samples varied from year to year, as total PAHs and total PCBs were found at levels as high as 3,286 ng/dry g and 3,760 ng/dry g, respectively. Sediment PAHs were predominantly high molecular weight PAHs. Total PAHs in water were as high as 11,900 ng/L, though most samples were considerably lower. PCBs were detected in water during only one sampling event. Analysis of fish tissue indicated that total PAHs were as high as 251 ng/g wet, while PCBs were below detection. Preliminary results of DNA adduct levels revealed that adducts were consistently higher in gills than in livers, and were also higher in salmon than in sculpin. Results confirm that biomarkers provide a sensitive measure of availability of contaminants in the water column. Possibly due to rapid metabolism, carcinogenic PAHs present in fish tissue were frequently close to or below detection limits. However, DNA adduct analysis appears to provide a more persistent marker of exposure.

1250 AN EXAMINATION OF HYPOTHESES FOR THE MECHANISM OF TRIBUTYLTIN-INDUCED IMPOSEX.

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This research used the mud snail *Ilyanassa obsoleta* to examine hypotheses for the mechanism by which tributyltin (TBT) induces imposex, the development of male sex characteristics by female neogastropods. One hypothesis is that TBT elevates free testosterone which then initiates the biological processes necessary for females to develop imposex. We tested whether TBT elevates free testosterone by inhibiting acyl coenzyme A:testosterone acyltransferase (ATAT) using in vitro assays with microsomes from *I. obsoleta*. Results showed that TBT inhibits ATAT activity at concentrations measured in snails from TBT-contaminated field sites suggesting that TBT elevates free testosterone in neogastropods by inhibiting their major regulatory process for maintaining free testosterone homeostasis. We addressed the assumption that testosterone contributes to reproductive tract recrudescence of neogastropods using radioimmunoassay to quantify seasonal testosterone levels and RT-PCR to identify an androgen receptor (AR) in *I. obsoleta*. Results showed that testosterone may have a role in male recrudescence; however, this putative activity is independent of a NR3C4-type AR. Collectively, these studies provided support for how TBT elevates free testosterone in neogastropods but cast doubt upon the validity of the hypothesis that the TBT-induced increase in free testosterone initiates a biochemical pathway resulting in imposex. Recent evidence suggests that the retinoid X receptor (RXR) participates in TBT-induced imposex. Accordingly, we hypothesized that the RXR may contribute to male reproductive tract recrudescence in neogastropods and thus would be expressed in concert with this phenomenon. We used real time RT-PCR to quantify seasonal levels of the RXR in *I. obsoleta*. Results demonstrated that RXR expression is associated with reproductive tract recrudescence in both sexes although the timing of recrudescence may dictate sex-specific development. Thus, retinoid signaling initiated by TBT during an inappropriate time in females may result in imposex.

S 1251 ENDOTHELIAL DYSFUNCTION: MORE THAN JUST A "NO NO" PHENOMENON.

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Various environmental and anthropomorphic toxins can cause direct or indirect injury to the vasculature, resulting in endothelial cell dysfunction. Endothelial dysfunction is thought to be an early marker of vascular injury and precedes overt macro- and microvascular pathology (e.g., atherosclerosis, peripheral artery disease, angiopoathy), and may even be a crucial factor in end-stage lung disease. Endothelial dysfunction has been measured in many forms such as decreased endothelium-dependent relaxation, increased expression of endothelial cell adhesion molecules (e.g., ICAM-1, VCAM-1,selectins, etc), increased circulating endothelial-derived products (e.g., sICAM-1, ET-1, vWF, etc…), and increased vascular permeability. Endothelial dysfunction is often linked to a generalized decrease in bioavailable or bioactive nitric oxide (NO), which could result from diminished eNOS expression, decreased eNOS activity due to decreased co-factor availability or protein uncoupling, and/or increased superoxide production, which can sequester NO to form peroxynitrite. In addition, as toxicants combine with pre-existing disease conditions to amplify vascular oxidative stress, endothelial dysfunction is typically evidenced by increased superoxide production, increased iNOS and cytokine expression, and increased protein modification by peroxynitrite, which could result in protein inactivation. Despite inconsistencies among methodologies across laboratories, and some variation among findings, data largely indicate that a variety of inhaled and ingested xenobiotics can adversely impact endothelial function, especially in the setting of pre-existing disease.

S 1252 MECHANISMS OF ENDOTHELIAL DYSFUNCTION: RELEVANCE TO INHALED PM.

S. Rajagopalan. *Ohio State University Medical Center, Columbus, OH.* Sponsor: D. Conklin.

A growing body of epidemiological data implicates particulate matter air pollution (PM) as yet another factor in the pathogenesis of cardiovascular disease. Loss of endothelium-derived nitric oxide (NO) is a central binding principle that has been implicated in the pathogenesis of diseases such as smoking related vascular disease,

atherosclerosis, hypertension and diabetes. The decline in NO bioavailability due to accelerated destruction by superoxide, is a common mechanism underyling endothelial dysfunction and may be relevant as PM is a facile generator of reactive oxygen species. While the precise locus of generation of radicals in response to PM is debated, there is reason to believe that the vasculature may be key to modulation of PM effects. Experiments in animal models and humans suggest that PM mediated effects on the vascular endothelial function are rapid and may occur within hours to days following exposure. Endothelial dysfunction mediated via PM may then play a critical role in setting the stage for vascular inflammation. Data from our laboratory suggest that acute and sub-acute exposure markedly alters endothelial function through NADPH oxidase dependent mechanisms altering the propensity to other insults such as high fat feeding and pressor agents. These findings have important implications for the synergistic effects of PM with other atherosclerotic risk factors.

S 1253 EFFECTS OF INHALED DIESEL EXHAUST ON VASCULAR OXIDATIVE STRESS AND ENOS FUNCTION.

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Environmental air pollution has been associated with increased hospital admissions and death due to heart failure. However, the exact mechanism(s) by which environmental air pollution affects the heart and vasculature is currently unknown. Heart failure and diabetic patients have pre-existing endothelial cell dysfunction and recent studies have found that exposure to environmental air pollution enhances vasoconstriction in these populations. Work in our laboratory has shown that diesel exhaust (DE), a major component of ambient urban air, could enhance vasoconstriction of mouse coronary arteries and, in a model of heart failure, increase thoracic venous pressure; these effects could be linked to enhanced vascular oxidant generation. Thus, we hypothesized that DE could enhance vasoconstriction in arteries and veins through uncoupling of endothelial nitric oxide synthase (eNOS). To test this hypothesis, we first exposed animals, whole body, to DE at 350μg/m^3 for 4h, after which mesenteric arteries and veins were isolated. Results from these experiments show that inhaled DE enhances the vasoconstrictive effects of ET-1 in veins more than arteries. L-NAME, an eNOS inhibitor, normalized the control vessels to the diesel exhaust vessels implicating an uncoupling of eNOS as a mechanism for enhanced vasoconstriction. These data suggest that exposure to inhaled DE may induce venous congestion in susceptible subjects through enhanced vasoconstriction due to vascular oxidative stress.

S 1254 REGULATION OF ENDOTHELIAL CELL ADHESION MOLECULES BY ACROLEIN, AN ENVIRONMENTAL ALDEHYDE.

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Acrolein, a toxic aldehyde generated during combustion of fossil fuels, is also generated endogenously during lipid peroxidation. Adhesion molecules ICAM-1 and VCAM-1 expressed on endothelial cells (EC) upon cytokine stimulation facilitate leukocyte adhesion and migration. In inflammatory diseases, the ectodomain cleavage of these adhesion molecules is augmented releasing soluble ICAM-1 (sICAM-1) and VCAM-1 (sVCAM-1) into the plasma. Mice exposed to acrolein (5 mg/kg by gavage) showed a significant (36 ±2%, P<0.001) reduction in the plasma levels of sICAM-1 at 4h after the exposure that persisted up to 24h (20±1%, P<0.001) when compared with water-fed controls. Human ECs were exposed to 0-10 μM acrolein for 1h in media without serum, allowed to recover for 24h, stimulated with TNF-α for 16h to induce adhesion molecule expression and cleavage, and then sICAM-1 and sVCAM-1 in the media were measured. A dose-dependent reduction in the levels of sICAM-1 (2-32%) and sVCAM-1 (17-63%) was observed. In such studies, TNF-α-induced JNK phosphorylation was diminished, but ERK 1/2 and p38 were unaffected by acrolein pretreatment. Monocyte adhesion to acrolein-exposed ECs was augmented (1.3-1.4 fold). However, acrolein did not affect basal sICAM-1 release in ECs virally-transfected with ICAM-1 (in the absence of cytokine). We have reported that ICAM-1 cleavage is mediated by the matrix metalloproteinase (MMP) sub-family, TNF-α converting enzyme (TACE) (J. Biol Chem. 2006; 281:3157). Expression of TACE mRNA (but not the levels of endogenous MMP inhibitors, TIMP-1, -2 and -3) and protein levels were marginally decreased in acrolein-exposed ECs. These results indicate that acrolein down regulates the shedding of ICAM-1 and VCAM-1 perhaps through JNK inhibition, and thus, augments leukocyte adhesion to EC.

S 1255 ULTRAFINE PARTICULATE MATTER INHALATION ATTENUATES MICROVASCULAR ENDOTHELIAL NITRIC OXIDE PRODUCTION.

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We have shown that pulmonary exposure to fine particulate matter (PM) impairs endothelium-dependent dilation in systemic arterioles. The purposes of this study were to: determine if PM size affects the severity of microvascular dysfunction, characterize alterations in endogenous nitric oxide (NO) production, and identify systemic mechanisms that may influence NO production after PM inhalation. Rats were exposed to fine or ultrafine TiO_2 via inhalation (primary particle sizes of ~1 μm, and ~21 nm, respectively) at depositions of 10-100 μg/rat. The spinotrapezius muscle was prepared for intravital microscopy 24 hrs after exposures. Intraluminal infusion of the Ca^{2+} ionophore A23187 was used to evaluate endothelium-dependent arteriolar dilation. Microvascular NO production was measured with a Clarke-Type electrochemical NO sensor. In control rats, A23187 infusion produced dose-dependent arteriolar dilations. In rats exposed to fine TiO_2, A23187 infusion elicited vasodilations that were blunted in proportion to pulmonary particle deposition. In rats exposed to ultrafine TiO_2, A23187 infusion produced arteriolar constrictions or significantly impaired vasodilator responses as compared to those observed in rats exposed to an identical pulmonary load of fine particles. Endogenous microvascular NO production was attenuated after ultrafine TiO_2 inhalation in a dose-dependent manner. Treatment with either 4-aminobenzoic hydrazide or apocynin partially restored NO production and normal microvascular function. These results are consistent with the hypothesis that systemic inflammatory mechanisms are active after PM exposure and such mechanisms disturb normal microvascular function.

S 1256 DIESEL EXHAUST EXPOSURE INCREASES BLOOD PRESSURE, SYMPATHETIC ACTIVITY, AND CORONARY ARTERY CONSTRICTOR FUNCTION.

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Some individuals experience acute cardiovascular events following exposure to air pollutants that may be triggered by vasospasm. Because the pollutant diesel exhaust (DE) has been shown to increase endothelin (ET) synthesis and sympathetic nervous system activity (SNSA), these events may be key to such adverse cardiovascular sequelae. If DE also augments coronary artery constriction to ET-1, this would exacerbate cardiac insufficiency following exposure to DE. We hypothesized that acute exposure to moderate levels of DE augments ET constrictor sensitivity and diminishes endothelium-dependent dilation. Rats were exposed to 5 hrs of DE (300 μg/m^3) or Filtered Air (FA). Arterial pressure (AP), electrocardiograms (ECG) and heart rate (HR) were recorded using telemetry. Coronary arteries were obtained after DE exposure to measure ET-1 constrictor responses and agonist-induced dilator responses. HR variability was derived from ECG (ratio of low to high frequency of transformed RR interval) and used as an index of SNSA. Tissue from the heart, intraseptal coronary artery, lung and aorta were collected immediately after DE exposure to determine ET-1/3 and $ET_{A/B}$ receptor mRNA using real time PCR. AP was elevated in rats exposed to DE compared to air exposed rats ($p<0.05$). SNSA was also greater in DE rats compared to controls. In isolated coronary arteries (inner diameter = 180 ± 11 μm) from FA and DE rats, DE augmented ET-1 constrictor responses and diminished acetylcholine-induced vasodilation compared to arteries from FA-exposed rats ($p<0.05$). Acute DE exposure did not alter ET peptide or receptor mRNA suggesting the increased constriction to ET-1 is caused by altered signal transduction rather than changes in receptor expression. In summary, DE exposure may increase cardiac work load concurrent with decreased efficiency of oxygen delivery. These combined changes may contribute to the detrimental cardiac effects of DE and the increased incidence of cardiac events observed following this exposure.

S 1257 ENDOTHELIAL DYSFUNCTION AND ENVIRONMENTAL ALDEHYDES: ACROSS SPECIES COMPARISONS.

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Reactive aldehydes are generated endogenously during lipid peroxidation and inflammation and are ubiquitous environmental pollutants. Protein adducts of acrolein and 4-hydroxy-trans-2-nonenal (4HNE) are increased in human atherosclerotic plaques, neuronal membranes of patients with Alzheimer's disease, and plasma of heart failure patients. To understand the potential vascular complications

of excessive aldehyde exposure, we studied acrolein-induced vasoreactivity in perfused rodent mesenteric bed, isolated rodent aorta, and human coronary artery bypass graft (CABG) blood vessels. Acrolein induced NO-dependent and NO-independent responses that were blood vessel and species specific. For example, acrolein-induced vasodilatation in rodent mesenteric bed was more robust and more sensitive than dilation induced by other aldehydes, 4-HNE, trans-2-hexenal, and propionaldehyde. However prolonged exposure to acrolein or the acrolein generator, allylamine, led to endothelial dysfunction and vascular toxicity in rodent aorta and CABG blood vessels. These data indicate that acrolein elicits potent vascular responses that transition from the physiological to pathophysiological, and thus, acrolein could contribute to vascular and endothelial dysfunction associated with pollutant exposure.

S 1258 ADENOSINE RECEPTOR INVOLVEMENT IN THE CONSTRICTOR RESPONSES FOLLOWING ULTRAFINE PARTICULATE MATTER EXPOSURE.

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Epidemiological studies correlate adverse cardiovascular events with elevated exposure to ambient particulate matter, and direct empirical observations reveal impairment of vasodilator mechanisms following exposure to particulate material. The present study investigated the adenosine-dependent vascular responsiveness of aorta of a murine model exhibiting discreet detrimental cardiovascular related endpoints following ultra fine particulate (UFPM) exposure. Mice were exposed to vehicle and 10, 30 and 100 μg doses of UFPM by intratracheal instillation. Twenty-four hours later, aortas were isolated and vascular reactivity to agonists and antagonists to specific adenosine receptors evaluated. UFPM exposure increased the vasoconstrictor response of denuded thoracic aorta to 10 μM adenosine by nearly 50%. At 100 μM adenosine, the relaxation response of aorta from UFPM exposed animals was significantly impaired with a maximal relaxation of only 16%. UFPM exposure elevated the aortic constrictor response and sensitivity through the A1 receptor agonist CCPA. Minimal/moderate effects were observed with the A2b or A3 receptor agonists NECA or CL-IB-MECA. UFPM exposure also exaggerated the constrictor response to adenosine in aortas preincubated with the A3 receptor agonist MRS1523. These results are consistent with the hypothesis that adverse cardiovascular events associated with particulate mater exposures involve altered adenosine receptor activity in the vasculature.

This research has been reviewed by the National Health and Environmental Effects Research Laboratory, US EPA, and approved for publication. Approval does not necessarily reflect EPA policy. Funding support impart by Philip Morris USA and International.

S 1259 NEUROTOXICANT-INDUCED ALTERATIONS IN DEVELOPMENTAL AND ADULT NEUROGENESIS: STRUCTURE-FUNCTION STUDIES AND CLINICAL RELEVANCE.

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The orderly formation of the CNS requires a multitude of complex, integrated and simultaneously occurring processes. Neural progenitor cells expand through proliferation, commit to different cell fates, exit the cell cycle, and generate different neuronal and glial cell types. New neurons then migrate to specified areas and establish synaptic connections. A similar neurogenic process has been identified in certain regions of the adult brain. Gestational, postnatal and/or adult exposure to well-known environmental toxicants, pharmacological agents and drugs of abuse produce persistent alterations in behavioral, cognitive, sensory and motor functions that likely reflect a disruption of these processes of CNS development. This symposium will provide a framework for understanding the orchestrated events of neurogenesis - especially the coordination of proliferation and cell fate specification - and will address the adverse effects of known neurotoxicants on neurogenesis in the brain and retina. The speakers will provide an integrated overview of these processes and molecular mechanisms in several different brain regions; present the molecular mechanisms of how low-level gestational lead exposure produces a selective increase in neurogenesis and cell proliferation of late-born retinal neurons (rods and bipolar cells), but not glial cells, which results in both a novel retinal phenotype and function in children and adult animals; discuss the mechanisms of how ethanol exposure during different life stages produces long-term changes in hippocampal neurogenesis, survival and cognitive function; describe the mechanisms of chronic lead exposure during early life on cell proliferation, survival and phenotypic differentiation of progenitor cells in the hippocampus and how these changes compromise synaptic plasticity and cognition.

S 1260 REGULATION AND SPECIFICATION OF NEUROGENESIS AND CELL FATE DURING CENTRAL NERVOUS SYSTEM DEVELOPMENT AND ADULTHOOD: AN OVERVIEW, CHALLENGES AND QUESTIONS.

M. A. Dyer. *Developmental Neurobiology, St. Jude Childrens' Research Hospital, Memphis, TN.* Sponsor: D. Fox.

Formation of the CNS and sensory systems involves the orchestrated regulation of neural progenitor cell proliferation, cell fate specification, migration and synaptogenesis. Because these processes occur simultaneously and defects in one influences the others, the mechanisms of each must be determined to understand CNS development. This talk focuses on the coordination of proliferation and cell fate specification that occur in neurogenesis. Several critical features must be characterized. First, the lineage relationships of different populations of progenitor cells (PC) in neural regions must be determined. Deregulated proliferation of progenitor cells affects cell type distribution differently, depending on whether the cell types are derived from a common PC or two PC populations that contribute to the mature tissue. Second, the birth order of cell types must be determined. That is, birth dates are used to generate models for the alterations in cell type distribution that result from deregulated neural PC and to determine whether a given protein regulates proliferation, cell fate specification or both. Third, genetic and molecular tools that can distinguish between intrinsic and extrinsic regulation of proliferation and redundancy versus compensation must be applied to individual neural PCs to obtain a complete picture of the coordination of proliferation and cell fate specification during CNS development. Additional key areas for research include determining: entrainment of intrinsic proliferation clocks by growth factors, neurotransmitters and neurotoxicants and how compensatory mechanisms are regulated at a genetic level. An increased knowledge of the coordination of proliferation and cell fate specification during early development and its alteration by exposure to heavy metals, ethanol, pesticides and drugs will improve our understanding of genetic and acquired behavioral, cognitive, sensory and motor deficits as well as childhood diseases. Supported by NIH, ACR, NSF, Res. to Prevent Blindness and Pew Foundation.

S 1261 GESTATIONAL LEAD EXPOSURE (GLE) UPREGULATES CELL CYCLE GENES AND PROLONGS RETINAL PROGENITOR CELL (RPC) PROLIFERATION RESULTING IN INCREASED ROD PHOTORECEPTOR AND BIPOLAR CELLS.

D. A. Fox. *College of Optometry, University of Houston, Houston, TX.*

GLE produces supernormal scotopic ERGs in children, monkeys and rodents. Previously we showed in adult mice that GLE produced a dose-dependent increase in the number of late-born retinal neurons (rods and bipolar cells), but not Müller glial cells. This study determined the molecular and cellular mechanisms underlying the novel retinal phenotype produced by GLE.

Female C57BL/6 mice were exposed to water or one of three different lead solutions throughout pregnancy and until postnatal day 10 (PN10): equivalent to human gestation period. Peak [BPb] ranged from 10-40 μg/dl. Microarray studies were conducted on E16.5, E18.5, PN2, PN6 and PN10 retinas using the Affymetrix GeneChip® Mouse Genome 430 2.0 Array. Results were validated by real-time Q-PCR and extended with SuperArray's cell cycle PCR array. Immunocytochemical and confocal (ICC) studies examined selected cell cycle and RPC markers. Cell counts were obtained using stereological techniques.

Functional classification of the microarray results revealed the up-regulation of several cell cycle genes. Q-PCR studies confirmed and extended the expression patterns of Ccnd1, Ccne1, Ccna1, Ccna2 and Ccnb1 and revealed the down-regulation of negative cell cycle regulator genes. ICC showed that developing GLE retinas had an increased number and prolonged expression of Ki67-, BrdU-, PH3- and cyclin D1-positive RPCs.

Microarray and Q-PCR studies showed that GLE up-regulated cell cycle genes. ICC studies confirmed and extended these results to reveal an increased and prolonged proliferation of RPCs. These findings show that GLE targets molecular sites in the developing retina that regulate RPC proliferation and neurogenesis to produce a persistent and selective increase in late-born retinal neurons. The results have relevance for the retinal ERG supernormality and neural deficits observed in children following GLE at the currently considered "safe level" of lead exposure.

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S 1262 ALCOHOL DYSREGULATION OF ADULT NEUROGENESIS: EFFECTS ACROSS THE LIFESPAN.

K. Nixon. *Pharmaceutical Sciences, University of Kentucky, Lexington, KY.* Sponsor: D. Fox.

The dogma that neurons are formed only during development has drastically shifted due to the discovery that neural stem cells form new neurons throughout life. Thus, only recently has the effect of alcohol on adult neurogenesis been described even though developmental alcohol exposure has long been known to alter neurogenesis. Although alcohol has distinct effects on the brain across the lifespan, excessive intake or exposure consistently impairs brain size, evidenced by a net decrease or loss of cells in many brain regions regardless of age of exposure. Cell death is commonly considered the mechanism of neurodegeneration, however the discovery of neural stem cells and the effect of alcohol on neural stem cells introduce a novel mechanism in which to consider alcohol-induced neurodegeneration. Similar to alcohol's effects on developing brain, the dose, duration and pattern of alcohol exposure are important factors in the effect of alcohol on adult neurogenesis. Exposure to alcohol in adolescence and adulthood inhibits cell proliferation during alcohol intoxication. Alcohol may also alter cell survival and neurogenesis though the effect depends on the timing, pattern, dose and duration of exposure. Recent work further shows that temporally- and regionally-specific cell proliferation events occur after alcohol-induced brain damage in both adolescent and adult rat models of an alcohol use disorder. These cell proliferation events reflect different populations of new cells, including new neurons. Comparing the similarities and differences in the effects of alcohol on the various components of neurogenesis across the lifespan highlight different mechanisms of alcohol effects on neurogenic processes: cell proliferation, migration, differentiation and survival. The mechanisms that underlie how alcohol exposure disrupts neurogenesis by decreasing progenitor cell proliferation, inhibiting cell survival and altering morphological maturation of newborn neurons as well as how these data implicate impaired hippocampal neurogenesis in alcohol-induced cognitive dysfunction will be discussed.

S 1263 ADULT NEUROGENESIS IN THE HIPPOCAMPUS: ALTERATIONS BY LEAD EXPOSURE AND LONG-TERM IMPLICATIONS FOR SYNAPTIC PLASTICITY AND COGNITION.

T. R. Guilarte. *Environmental Health Sciences, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD.*

Exposure to environmentally relevant levels of lead during development is known to cause deficits in synaptic plasticity and cognitive function in experimental animals and humans. Our laboratory has previously shown that developmental lead exposure produces deficits in spatial learning performance in rats. Other evidence suggests that spatial learning performance is associated with the level of granule cell neurogenesis in the dentate gyrus of the rat hippocampus. Further, modulation of neurogenesis by endogenous and external factors can alter synaptic plasticity and cognitive function. The goal of this presentation is to describe studies in which exposure to environmentally relevant levels of lead during development alters granule cell neurogenesis and morphology in the adult rat hippocampus. We show that lead-exposed rats have decreased granule cell proliferation and survival relative to control animals. Morphologically, newly born granule cells from lead-exposed rats displayed aberrant dendritic morphology with decreased dendritic length. We also found reductions in the mossy fiber terminal fields representative of decreased innervation to the CA3 region. We propose that lead-induced changes in granule cell neurogenesis and morphology may be linked to deficits in synaptic plasticity and spatial learning documented in lead-exposed animals. Non-pharmacological therapeutic strategies to reverse the detrimental effects of lead on granule cell neurogenesis and morphology will be discussed.

S 1264 NOVEL BIOMARKERS OF DRUG-INDUCED TOXICITY: OUTCOMES OF PREDTOX AND THE PREDICTIVE SAFETY TESTING CONSORTIUM.

D. Robinson-Gravatt[1] and I. Y. Rosenblum[2]. [1]*Drug Safety R&D, Pfizer, Groton, CT and* [2]*Non-clinical Drug Development, Kyowa Pharmaceutical Inc., Princeton, NJ.*

There is general agreement regarding the potential for safety biomarkers to provide earlier and more sensitive or specific indicators of preclinical and clinical safety, to improve understanding of mechanisms of toxicity, and to speed up and/or reduce the cost of preclinical drug safety evaluation. It is also increasingly recognized that the path to establishing new biomarkers is time and resource intensive, and will be more efficiently progressed through collaborative approaches. To this end, several consortia have been created to jointly develop the necessary data to advance novel safety biomarkers. The European Commission's PredTox Project, involving pharmaceutical companies, universities and regulatory agencies in Europe has focused on the usefulness of "-omic" technologies in pre-clinical safety testing, and the value of combining results from omics technologies with the results from more conventional toxicology methods. Likewise, the Predictive Safety Testing Consortium (PSTC) has been established as a collaboration between the pharmaceutical industry, the FDA, and the Critical Path Institute, with the mission to efficiently qualify new biomarkers of drug-induced toxicity for application in drug development and regulatory decision-making. Both of these efforts have made use of the combined resources of the participants to rapidly discover (PredTox) and qualify (PSTC) new safety biomarkers. The involvement of regulatory scientists assures progress toward predictive biomarkers that are relevant for preclinical and investigative clinical application and regulatory decision-making. The Symposium will showcase progress of the working groups of PredTox and the PSTC toward novel assays of nephrotoxicity, hepatotoxicity, vascular injury, and non-genotoxic carcinogenicity, as well as an overview of the regulatory review process for these assays within the Pilot Process for Biomarker Qualification initiative at the FDA.

S 1265 FIRST REGULATORY SUBMISSION OF NEWLY QUALIFIED ACCESSIBLE BIOMARKERS OF NEPHROTOXICITY *VIA* A NEW ROLLING QUALIFICATION PROCESS.

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Accessible biomarkers for monitoring early onset of drug induced kidney pathology in drug trials are lacking primarily due to their late response and low sensitivity. Serum creatinine, BUN and measurements of GFR are all common estimates of kidney function but do not generally respond until a significant part of the kidney and its function has been altered. The development of drug candidates presenting kidney toxicities in GLP toxicology studies conducted to support the safe conduct of clinical trials will often be abandoned because of our poor ability to monitor for drug-induced kidney pathologies with precision. This happens for example when safety margins are not high or even when findings appear in only one single species and human irrelevance cannot be adequately assessed.
The C-Path PSTC Nephrotoxicity Working Group has submitted a Voluntary eXploratory Data Ssubmission package to FDA and EMEA reviewers with data from 34 rat studies supporting the utility as regulatory decision making and drug development tools of the functional urinary biomarker albumin, and the injury biomarkers clusterin, trefoil factor 3 and Kim-1 which outperformed BUN and serum creatinine for monitoring the very early onset of specific kidney tubular pathologies at the point when toxicities are very mild. Cystatin C, beta-2 microglobulin, total protein showed significant value over BUN and serum creatinine as functional biomarkers for monitoring glomerular injuries. Additional markers are under evaluation on remaining urine samples from these same studies and from additonal studies (NAG, GSTa, GSTm, RPA1, LCN2, osteopontin, osteoactivin, uromodulin). Lessons learnt from this first submission of markers via a new rolling qualification process will be discussed. Understanding each biomarker's comparative strengths and weaknesses and thereby appropriately matching performance expectations to specific drug development situations are essential for these tools to be able to promote drug development and drug safety monitoring.

S 1266 NOVEL BIOMARKERS OF HEPATOTOXICITY.

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Within the Predictive Safety Testing Consortium, the Hepatotoxicity Work Group has identified key opportunities for biomarkers with improved sensitivity and specificity, including markers for hepatocellular injury, biliary epithelial hyperplasia, and hepatic fibrosis. Cross-validation work is progressing on a collection of rat serum enzyme activities that includes paraoxonase-1, malate dehydrogenase, purine nucleotide phosphorylase, glutamate dehydrogenase, and alpha-glutathione S-transferase ELISA. The cross-validation involves the establishment of assay (technical) comparability across several laboratories, understanding analyte stability, normal reference ranges, and the procurement and analysis of serum or plasma samples from studies employing a range of hepatotoxic, non-hepatotoxic and control articles. Biomarker performance is being assessed both technically (intra- and inter-assay precision, accuracy, reproducibility) and biologically. In particular, how do novel biomarkers perform relative to existing biomarkers regarding onset of injury, severity of injury, dose-response, its dynamic range and specificity for liver injury vs. other organ injury. Another area of cross-validation is based on genomic markers of rat hepatotoxicity (fibrosis and bile duct hyperplasia). Several partners have established rat liver toxicogenomic data bases with a wide variety of reference toxi-

cants that can enable relatively rapid cross-validation "in silico" using these preexisting data. For both types of biomarkers, the efforts have been facilitated by adopting a standardized histopathology lexicon and articulated goals for both the technical and biological aspects of cross-validating novel biomarkers.

S 1267 QUALIFYING NOVEL BIOMARKERS OF VASCULAR INJURY.

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In preclinical toxicology testing, drug-induced vascular injury (DIVI) has been observed with a number of structurally and pharmacologically diverse compounds. Although several mechanisms of vascular toxicity have been proposed, the mechanism(s) by which these drugs damage blood vessels is not known, nor is the relevance of preclinical DIVI to humans. In addition, there are no specific biomarkers that can be used preclinically or clinically to either predict or diagnose DIVI. As a result, the development of important new drugs can be significantly delayed or terminated when DIVI is encountered. Because this is an issue that has been encountered by many different pharmaceutical companies, and one that is too complex to be solved by individual companies working in isolation and with limited resources, the Vascular Injury Working Group (VWG) of the Predictive Safety Testing Consortium (PSTC) was formed to identify non-invasive biomarkers of DIVI and develop a translation strategy to determine the relevance of preclinical findings to humans. VWG members have shared detailed information on individual biomarkers under development, including experimental protocols, assay performance characteristics, and qualification results. In addition, the PSTC agreement has enabled VWG members to exchange several hundred rat and dog serum samples for the generation of preclinical cross-qualification data. A particularly important objective of the VWG is to define a strategy for the clinical qualification of candidate preclinical biomarkers. Collaborations with internal and external partners for access to clinical samples from patients with different vascular diseases are actively being pursued by the VWG.

S 1268 VALIDATION OF RAT HEPATIC mRNA SIGNATURES FOR NONGENOTOXIC HEPATOCARCINOGENS.

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Two-year mouse and rat carcinogenicity bioassays and six-month transgenic mouse studies currently serve as the accepted benchmark for assessment of the carcinogenicity of novel pharmaceutical agents. Generally, genotoxic compounds (mutagens) are identified and triaged early from compound pipelines. However the discovery of unexpected, presumed non-genotoxic, carcinogenicity late in Development may prevent potentially good medicines reaching patients for years while risk assessment work is undertaken to qualify human risk. Tools for the early identification of nongenotoxic carcinogens are lacking or imprecise at best. The carcinogenicity working group (CWG) of the C-Path Predictive Safety Testing Consortium (PSTC) has selected genes, from published high density array work, determined to be of high predictive value in the early recognition of nongenotoxic hepatocarcinogenicity and consolidated this list for refinement and qualification as a gene signature to predict a compounds potential to be a nongenotoxic hepatocarcinogen. Rat liver mRNA from studies of a wide range of carcinogenic and non-carcinogenic compounds carried out at by the participating companies enabled a comprehensive evaluation of the gene expression signature. To ensure platform-independent utility, mRNA for these genes was assayed by realtime quantitative PCR, and a final signature was rederived from genes with confirmed expression. The robustness and potential utility of this new signature will be discussed.

S 1269 -OMICS IN HEPATOTOXICITY PREDICTION.

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Sponsor: F. Pfannkuch.

The attrition rate of drug candidates due to late-breaking findings in preclinical safety studies is high. Improved prediction of drug toxicity and understanding of key mechanisms of toxicity through incorporation of new concepts and technologies may aid regulatory decision making and decrease the cost of drug development. To achieve these goals, the PredTox project, a collaborative effort by 15 pharmaceutical companies, 2 SMEs and 3 universities was initiated to assess the value of combining -omics technologies with conventional toxicology methods for improved prediction of toxicity and to identify novel biomarkers of (nephro-) and hepatotoxicity. Comprehensive data sets were collected from in vivo experiments in which rats were treated with the model compound troglitazone or one of 12 proprietary compounds that previously failed during drug development partly due to toxic effects in the liver. Liver, kidney, blood and urine were analyzed by a combination of -omics techniques, including toxicogenomics(Affymetrix), toxicoproteomics and metabolic profiling. Clinical chemistry and histopathology were assessed to provide phenotypic anchoring. Data sets were entered into an integrated database for in-depth cross-platform and cross-study data analysis. Hypertrophy, hepatocellular necrosis and biliary cell hyperplasia/necrosis were the most frequent histopathological alterations observed. For most compounds, treated animals were separated from control animals in a dose-dependent manner by principal component analysis. In several cases, -omics technologies were able to identify changes indicative of a toxic response before alterations were detected by conventional endpoints, suggesting that -omics technologies may contribute to improved prediction of hepatotoxicity and biomarker identification. Observations regarding synergies between technologies for improved MOA/MOT investigations will be presented.
The contribution of all members of the PredTox Consortium (www.innomed-predtox.com) is gratefully acknowledged. The InnoMed Project is supported by partial funding by the Sixth Research Framework Program of the European Union (LSHB-CT-2005-518170).

S 1270 AN INTEGRATED –OMICS APPROACH TOWARDS A BETTER UNDERSTANDING OF DRUG-INDUCED NEPHROTOXICITY AND USEFUL BIOMARKERS.

F. Staedtler. *Biomarker Development, Novartis Pharma AG, Basel, Switzerland.*

In order to increase early attrition in preclinical pharmaceutical drug development, the new omics technologies have been assessed by investigational toxicologists and technology experts. The InnoMed PredTox consortium with participation of 3 academic institutions, 2 technology vendors and 15 major pharmaceutical companies, has focused on a systematic and integrated -omics approach assessed against conventional toxicology assessment in the rat. The goal was to obtain better insight into drug-induced organ toxicity and to confirm and detect early molecular biomarkers of organ toxicity. As a unique feature, the consortium members have selected 14 proprietary compounds, troglitazone that had been withdrawn from the market, and a reference nephrotoxicant, gentamycin. The drug development program of the proprietary compounds was abandonned for toxicity reasons at different development stages. The presentation will summarize and discuss the results obtained so far by the InnoMed PredTox consortium with particular emphasis on nephrotoxicity and potential ways forward towards a further validation and useful application of the results including the candidate biomarkers.

S 1271 A YEAR OF BIOMARKER QUALIFICATION SUBMISSIONS THROUGH THE PILOT PROCESS FOR BIOMARKER QUALIFICATION AT THE FDA.

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Sponsor: I. Rosenblum.

The FDA has recently developed a Pilot Process for Biomarker Qualification, with a goal of qualifying novel biomarkers in the context of drug development and regulatory review. Over the past year this pilot process has been tested with submissions providing evidence for the qualified use of certain nonclinical and clinical biomarkers. Results from these submissions reveal a number of critical issues that need to be addressed for successful qualifications, including: mechanistic correlations of the biomarkers, biomarker accessibility and stability in bodily fluids, baseline levels of the biomarkers in control samples, and potential interferences in the chemical matrix of the measurement. Critical questions related to qualification study design include: the number of analytical and biological replicates, the relevance of the nonclinical animal models, and potential clinical constraints. How these questions are addressed will significantly impact the complexity of the qualification study designs. Consortia such as the PSTC provide the leadership for organizing and coordinating cross-qualification studies as well as confirmation of biomarker data across multiple test sites. Regulatory structures supporting biological qualification encompass the biological, clinical, analytical and statistical expertise needed to assess submissions of qualification data. These structures also reflect ownership by the regulatory reviewers who will eventually be responsible for review of INDs, NDAs or BLAs containing data in support of qualified biomarkers.

W 1272 DERMAL TOXICOLOGICAL ASSESSMENT OF NANOMATERIALS AND NANODEVICES.

D. Hobson[1] and R. Osterberg[2]. [1]*H&H Scientific LLP, Boerne, TX and* [2]*Aclairo PDG Inc., Vienna, VA.*

Developments in nanotechnology are creating a rapidly increasing number and variety of engineered nanomaterials and nanodevices with potential for dermal applications in topical drug and cosmetic product formulations. Dermal exposures associated with various industrial applications, occupations and environmental releases related to these developments may also occur. The small size and large surface area of nanomaterials provides for unique properties of these materials in addition to their chemical composition that allow them to advance or enhance technology in an expanding number and variety of applications such as consumer goods (foods, household goods, cosmetics, textiles, etc.), drugs and drug delivery systems and diagnostic systems. Dermal exposures to nanomaterials have potential to enhance or modify the efficacy of therapeutics and skin protectants as well as show toxicity that is differential to that expected due to chemical composition and from materials that normally could not gain entrance to the body via dermal routes that are relatively unique to nanomaterials. While some nanomaterials appear to be safe at this time, it is becoming evident that nanomaterials may become differentially activated biologically and exhibit pharmacologically and toxicologically significant properties that are not evident in micro or macroparticulate forms for the same materials that must be taken into account in the design of dermal toxicological studies. The results of different investigations being conducted internationally clearly indicate that the biological activity and pharmacokinetics of nanoparticles are different from non-nanoparticles. These differences can change the protein binding, cellular uptake, distribution and the pathologies of materials for which toxicities are already understood and may make prediction of dermal toxicity even more difficult and complex than presently exists with current testing methodologies. Current data demonstrate that nanomaterials should be tested using specific modifications to existing procedures or using new methods specifically developed to optimize the assessment of toxicological effects of these nanomaterials.

W 1273 SAFETY CONSIDERATIONS FOR THE DEVELOPMENT OF NANOMATERIALS AND NANODEVICES WITH TOPICAL EXPOSURE POTENTIAL.

D. Hobson. *H&H Scientific LLP, Boerne, TX.*

As engineers and materials scientists work expeditiously seeking the promise of the future industrial revolution and marketability of new engineered nanomaterials, nanodevices and new technologies derived from their use, safety considerations must become an essential part of this increasing discovery and early stage development activity. Although criticism has been levied at regulatory bodies worldwide, it is already evident that new nanomaterials have entered consumer markets and the environment without causing recognized adverse effects but it is important to realize that the long term risks and environmental impacts of many of these materials are still unknown as are the toxicokinetics and histopathological effects of repeated exposures via various routes including the dermal route. Topical products such as sun protection and wound healing formulations containing nanomaterials have been on the forefront of some of this controversy with divided perspectives from scientists and consumers. Occupational exposures to dusts also are a growing concern from topical exposures to nanomaterials as well as the potential for exposures to engineered nanomaterials from environmental sources including the discharge of nanosilver particles from different antimicrobial uses. This presentation will provide an up to date overview of the current state of toxicological science from a global perspective to address concerns about topical exposures to nanomaterials. Furthermore, it will discuss current strategies for the screening and testing of engineered nanomaterials to evaluate their topical safety.

W 1274 FDA PERSPECTIVE ON TOPICAL NANOMATERIALS IN COSMETICS.

R. Bronaugh. *FDA, College Park, MD.*

Topical nanomaterials are increasingly being used in cosmetic products. Liposome technnology has been used for many years in cosmetics but in recent years smaller, nanoscale liposomes have been produced by filtration through nanosized pores. An oil-in-water nanoemulsion contains suspended oil droplets or vesicles in the nanometer range that can encapsulate ingredients and offer improved texture. Aqueous suspensions of solid lipid nanoparticles can be prepared that may provide advantages in stability and delivery capabilities. Nanoparticles can be used to stabilize ingredients that are sensitive to air and light exposure. However, if this technology is used for increased delivery of topically applied ingredients, safety concerns could develop if the ingredients have biological activity.

We have begun to examine the ability of liposome formulations to enhance the in vitro percutaneous absorption of ingredients applied to skin in diffusion cells. Liposomes made with egg phosphatidyl choline and the surfactant Tween 80 appeared to facilitate some penetration of the lipophilic dye (1,'-dioctadecyl-3,3,3',3'-tertramethylindocarbo-cyanine perchlorate, DiI) into the viable tissue of hairless guinea pig skin. Analysis of the structure of the vesicles by Small-Angle Neutron Scattering (SANS) indicates that micelles of Tween 80 are formed in the formulations with the higher concentrations of Tween 80 where skin penetration was observed.

Other studies have been conducted in vitro with human skin and two quantum dot nanoparticles used as model compounds because of their relative stability and strong fluorescence. Quantum dots fluorescing at either 621 nm (37 nm hydrodynamic diameter and a PEG coating) or 655 nm (20 nm hydrodynamic diameter and an amino PEG coating) were found mostly in the stratum corneum at the end of the absorption studies. However some studies with the 655 quantum dots showed small amounts of penetration into the viable epidermis.

Further studies are needed with other nanoparticles to help understand the effects of particle size, surface characteristics, and other properties on skin penetration.

W 1275 CHPA PERSPECTIVES FOR NANOTECHNOLOGY DERIVED CONSUMER PRODUCTS.

H. Schneider. *Consumer Healthcare Products Association, Washington, DC.*

A variety of different healthcare products are currently categorized under the ill-defined term "nanotechnology". Sunscreens using nanoparticles can be found under this category, as well as advanced drug delivery systems, wear-resistant coatings of implants, and medical diagnostics. A generalized approach is therefore not feasible, and each product and therapeutic modality should be assessed on a case-by-case basis. This presentation will argue that the relationship between the structure of a material (with size being only one factor) and its chemical and physical properties should be investigated before a safety-testing strategy is developed.

W 1276 STUDY DESIGN CONSIDERATIONS FOR THE EVALUATION OF SKIN EXPOSURES AND BIODISTRIBUTION TO ENGINEERED NANOPARTICLES.

N. A. Monteiro-Riviere. *North Carolina State University, Raleigh, NC.*

Engineered nanomaterials are rapidly being developed and used for applications with potential for skin exposure, either intentionally in topical drug or cosmetic formulations or unintentionally as exposure to raw materials during manufacturing. Current studies with nanomaterials including quantum dots, fullerenes, as well as single- and multi-walled carbon nanotubes show that study design factors, especially the physicochemical properties of nanomaterials can play a significant role in the effects and particle dispositions observed from skin exposure studies. Dose preparation, vehicle and surfactant effects can occur. . Biomarker screenings may be altered in response to dispersant selection, type of nanomaterial or other factors that can impact tissue distribution. These effects and ultrastructural results in vitro cell culture, flow-through diffusion cells, and in isolated perfused skin can be demonstrated. We have shown that 565 and 655 QD can penetrate skin in flow-through diffusion cells. QD 621 with different surface coatings were intra-arterially infused into the isolated perfused porcine skin flap (IPPSF) at four concentrations for 4 hr (dose phase), followed by an additional 4 hr perfusion (wash-out phase). Skin was cryosectioned and imaged by laser scanning confocal microscopy. The images revealed epidermal blistering caused by QD aggregation and localization were noted within the endothelium of the capillaries, muscle, and subcutaneous tissue. The frequency of the QD decreased with a decrease in QD concentration and from the proximal to the distal end of each IPPSF. These results indicate aggregation of infused QD in the skin vasculature with concomitant blistering of the skin. This is important for risk assessment and drug delivery, since it shows the QD not specifically targeted for medical applications can aggregate in multiple tissues and cause pathological effects. Future studies on uptake and the cutaneous distribution are planned. (Supported by US EPA-STAR Program Grant RD-83171501 and National Academies Keck Futures Initiative.)

W 1277 ASSESSMENT OF THE DERMAL PENETRATION AND TOXICITY OF NANOPARTICULATE METALS.

S. M. Roberts, K. W. Powers and S. C. Wasdo. *University of Florida, Gainesville, FL.*

Study design factors can play a significant role in determining the outcome of studies of dermal disposition and toxicity of metal nanoparticulates. The properties of metal nanoparticulates can change substantially during preparation and delivery to

the skin. Therefore, it is important to characterize the nanoparticulates with respect to key physicochemical properties (e.g., particle size distribution, surface charge, effective surface area) as close to actual test conditions as possible. Examples will be presented that illustrate the challenges of characterizing metal nanoparticulates in biological environments. The choice of vehicle can have profound effects on the integrity of the skin, in addition to influencing the physical state of the metal nanoparticulate. Proper dosimetry can depend upon both the mechanism of toxicity and the manner in which the material enters the skin. Theoretically, metal nanoparticulates can penetrate the skin and can also serve as source of soluble metal species on the skin surface. Distinguishing between metal particulates and particulate-derived soluble species in the skin requires microscopic examination that is time-consuming and technically demanding. The ability of metal nanoparticulates to produce soluble species on the skin surface is determined to a large extent by particle's chemical composition and surface area, as well as the composition of the vehicle and localized skin conditions (e.g., pH, ionic strength). Based on our initial findings with selected metals, dermal bioavailability of metal nanoparticulates appears to be low, although this does not preclude direct toxicity of metal nanoparticulates on the skin or the possibility of systemic toxicity mediated through immune mechanisms, such as sensitization. Approaches in assessing these potential toxic endpoints for metal nanoparticulates include both traditional and novel techniques.

W 1278 HOST SUSCEPTIBILITY AND CHEMICAL SAFETY TESTING: NEW APPROACHES TO ESTIMATE RISKS IN THE HUMAN POPULATION.

I. Rusyn[1] and J. E. French[2]. [1]*Environmental Sciences and Engineering, The University of North Carolina at Chapel Hill, Chapel Hill, NC and* [2]*Laboratory of Molecular Toxicology, NIEHS, NIH, Research Triangle Park, NC.*

The focus of classical toxicology is on dose-response relationships and mode-of-action paradigms. Yet, the effects of many drug and environmental chemical exposures are context dependent, with genetic diversity between individuals a major variable that is usually overlooked in safety testing. Recent advances in our basic understanding of the genetic diversity of the human genome, as well as genomes of model organisms used in toxicity and safety assessment testing, not only provide an improved basis for species comparisons, but also serve as a lead into designing new approaches for understanding and estimating the risks in a genetically diverse population. The presentations and discussion in this session will highlight 1) new initiatives being considered for improvement of chemical testing at the National Toxicology Program based on host susceptibility and high throughput testing systems; 2) the use of genetic differences between isogenic mouse strains to develop knowledge applicable to human populations studies with regard to assessment of organ-specific toxicity of xenobiotics; 3) experimental models that explore the relationship between highly conserved mouse and human genes that affect responses to environmental agents; 4) the utility of densely-genotyped mouse and human lymphoblast cell lines to discover genetic polymorphisms that may be critical to population-wide responses to environmental agents; and 5) a perspective for introducing the aspect of genetic diversity and the relative value into drug safety evaluation of pharmaceuticals. Improved approaches and methods based on the rapid developments in genetics and genetic tools should lead to improvements in the use of experimental models for the identification of the genes and their allelic forms associated with phenotypes of interest for extrapolation to humans and to aid in the identification of orthologous human genes in genetically diverse human populations.

W 1279 GENETIC VARIATION AND INDIVIDUAL SUSCEPTIBILITY TO ENVIRONMENTAL EXPOSURE AND DISEASE.

J. E. French. *Host Susceptibility Branch, NIEHS, NIH, Research Triangle Park, NC.*

The NTP Host Susceptibility Rapid Access to Gene-Environment Interactions (RAGEI) program is designed to accelerate investigation of the relationship between exposure to environmental toxicants associated with human disease and the development of quantitative measures of toxicity and disease using genetically diverse experimental animal models. This multidisciplinary research approach will emphasize collaborative research and partnerships between the National Toxicology Program (NTP) and extramural and intramural investigators. Using the significant genetic diversity captured within multiple isogenic or outbred laboratory and/or wild-derived mouse strains or other animal or genetic models, as warranted, our goal is to model and predict potential human population-level ranges of response to toxicant exposure and to link environmental exposure and disease outcome. To achieve this goal we will support identification and functional characterization of specific genes, genotypes (haplotypes) and their allelic variants that are associated with individual differences in response to toxicant exposure. These data will allow the NTP and its extramural and intramural research partners to use comparative genetic analysis of discovered susceptibility genes to identify risks specific to susceptible human populations harboring structural or functional genetic variations in orthologous genes and pathways, many of which have been cataloged in the NIEHS Environmental Genome Project. Completion of the sequencing of the C57BL/6J isogenic mouse genome and resequencing (dense SNP genotype maps) of 15 isogenic laboratory and wild-derived mouse strains has demonstrated that genetic diversity in the combined mouse populations is at least equivalent to that of the human population. Determination and demonstration of the comparative functional relationships in gene-environment interactions relative to the human genome is now required to rapidly develop strategies and policies for prevention and mitigation of environmental exposure related disease.

W 1280 MOUSE MODELS FOR HUMAN POPULATIONS AND DISEASE: BUILDING A HUMAN-TO-MOUSE-TO-HUMAN CHEMICAL SAFETY TESTING PARADIGM.

D. Threadgill[1, 2], I. Rusyn[2] and A. Hege[2]. [1]*Genetics, University of North Carolina, Chapel Hill, NC and* [2]*Curriculum in Toxicology, University of North Carolina, Chapel Hill, NC.*

Although human populations have extensive genetic heterogeneity, this is rarely considered when evaluating chemical safety. However, new genome re-sequencing data in mouse has revealed that mouse populations can be designed to model complex human populations. By using exposures to different chemicals on a panel of mouse inbred strains that model genetic diversity in human populations, we show that underlying genetic variation has a significant impact on the molecular and phenotypic endpoints and adds an important dimension to toxicology. Specifically, we validate a strategy using a Mouse Model of the Human Population (MMHP) to identify genetic polymorphisms and novel mechanisms contributing to xenobiotic-induced liver injury. As a test case of this approach, we identified the genetic causes of acetaminophen-related liver injury by employing whole-genome haplotype association analysis in the MMHP and discovered that polymorphisms in Ly86, Cd44, Cd59a, and Capn8 are candidate genes. We then confirmed that variation in the orthologous genes is associated with susceptibility to acetaminophen in humans. These studies support the idea that a genetically diverse MMHP can be useful as a model to understand and predict adverse toxicity in humans.

W 1281 THE GENETIC BASIS FOR SUSCEPTIBILITY TO ENVIRONMENTAL LUNG DISEASES IN MOUSE AND HUMANS.

S. R. Kleeberger. *NIEHS, Research Triangle Park, NC.* Sponsor: J. French.

Epidemiological studies have associated exposures to outdoor and indoor pollutants with increased morbidity and mortality in urban areas throughout the U.S. and other countries. Thus, identification of the intrinsic and extrinsic factors that influence susceptibility to the pulmonary response to environmental exposure is important. Over the last several years, considerable effort has focused on understanding the role of genetic background as a susceptibility factor in environmental lung disease, particularly on genetic polymorphisms in the oxidative stress pathway. This talk will describe our current state of knowledge of genetic factors controlling susceptibility to oxidative stress and disease. The role of polymorphisms in the NRF2 transcription factor gene and the pathogenesis of acute lung injury will be presented as an example of the utility of genetic approaches to understand disease susceptibility.

W 1282 GENETICS OF VARIATION IN HUMAN GENE EXPRESSION AT BASELINE AND IN RESPONSE TO GENOTOXIC AGENTS.

V. G. Cheung. *Departments of Pediatrics and Genetics, University of Pennsylvania, Philadelphia, PA.* Sponsor: J. French.

Individuals are exposed to genotoxic agents such as radiation in clinical settings and through the environment. Individuals differ in sensitivity to radiation; however, except for rare cases of radiosensitivity syndromes such as Ataxia Telangiectasia, little is known about the basis of radiosensitivity. Limited by the lack of family material, genetic studies of sensitivity to toxins have been very difficult. We are addressing this problem by developing a cell-based genetic approach to identify regulators of cellular response to toxins. Expression level of genes underlies the phenotypic manifestation of diseases and response to environmental and therapeutic compounds. The expression levels of genes at baseline and in response to stimuli vary among individuals. We have previously shown that there is a genetic component to this variability. Using genetic linkage and association analyses, we have identified determinants that influence individual variation in gene expression. We have now extended those analyses to identify genetic determinants of response to radiation. We have

measured the expression levels of several thousand genes in lymphoblasts of members of the three-generation CEPH families at baseline and in response to radiation exposure. We treated the expression levels as quantitative traits, and carried out genome-wide linkage analyses to determine the chromosomal locations linked to these expression phenotypes. The results showed significant evidence of linkage for several thousand expression phenotypes (p < 3 x 10-5). For some phenotypes, the evidence of linkage is far beyond this threshold. The results are allowing us to uncover the global landscape of variation in gene expression and of transcriptional control in cells at baseline and in irradiated cells and the genetic basis for the response to genotoxin exposure.

W 1283 UNDERESTIMATING THE IMPACT OF RODENT GENETICS ON PHARMACEUTICAL SAFETY ASSESSMENT.

F. D. Sistare. *Safety Assessment, Merck & Co., Inc., West Point, PA.*

Animal toxicology findings can lead to a high rate of attrition at both early and late stages of drug development, and are often insufficiently understood to be able to make timely and accurate assessments of human relevance. Strong toxicity signals seen in only a few animals of a study group, differences in toxicities between test species, and differences in toxicity sensitivities between strains of a given species all point to underlying genetic differences and raise questions regarding human relevance. Examples will be presented 1) of individual rodent susceptibilities to treatment related toxicities that are clearly attributable to identified polymorphic differences in drug metabolizing enzymes and drug exposure levels, and 2) of genetic differences between mice that manifest in striking differences in xenobiotic tumor susceptibility. These types of observations suggest that we may be missing opportunities for a genetics toolbox to advance investigative toxicology approaches to better understand the human relevance of certain animal toxicology findings. A brief review of these examples will launch a roundtable discussion into both the merits of deploying genetic approaches to explain individual animal differences that are likely irrelevant to humans, as well as the merits of investigating rodent strain differences to reveal potentially human relevant population susceptibility risks.

W 1284 MOLECULAR MECHANISMS AND MOLECULAR BIOLOGY OF METAL CARCINOGENESIS: CHEMISTRY, MOLECULAR GENETICS, EPIGENETICS, AND ABERRATIONS IN GENE EXPRESSION.

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Specific compounds of the metals, nickel, chromium, cadmium, and lead, and the metalloid, arsenic, are human carcinogens. Mechanisms of metal/metalloid carcinogenesis include uptake of ions into cells by cellular transport systems - anion transport carriers for soluble Cr(VI)compounds or phagocytosis for insoluble Ni or Cr compounds, followed by dissolution of metal ions inside cells. Intracellular Ni(II) and Cr(III) ions bind to proteins. Reduction of protein-bound metal/metalloid ions [Ni or Cr(VI)ions] inside cells leads to oxygen radical generation, and 8-OH-dG, which leads to mutagenesis and gene amplification. Low doses of Pb, Cd, and As also interact to induce oxidative protein damage and 8-hydroxy-dG damage in DNA. Studies of metal ions in yeast cells indicate that many metal ions cause substantial global de-regulation of gene expression in yeast. In mammalian cells, Ni(II)ions cause chromosome aberrations and amplification of onocogenes, leading to over-expression of proto-oncogene mRNAs and proteins and expression of mutated oncogene protein products. Ni(II)ions also cause chromatin condensation and inhibition of histone demethylases. This then leads to DNA methylation and silencing of many genes, including tumor suppressor genes. The combinationn of activation of oncogenes and inactivation of tumor suppressor genes by metal ions, leads to loss of transcription of genes controlled by actively transcribed tumor suppressor genes and aberrantly high expression of genes controlled by proto-oncogenes, in mammalian cells. This results in global disruption of gene expression in cells. Approximately 150 genes are aberrantly expressed in Ni(II)transformed cell lines. The resultant global disruption in cellular gene expression leads to neoplastic transformation of cells, hence carcinogenesis.

W 1285 MOLECULAR TOXICOLOGY OF TRANSITION METALS: FROM YEAST TO MAN.

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Organisms are continuously exposed to transition metals. Exposure to higher than physiological levels of essential metals or to non-essential metals may lead to pathologic conditions. To avoid metal-induced cytotoxicity, sophisticated mechanisms for regulating the intracellular concentrations of metals and repairing metal-induced damage have evolved. Since many of these responses are conserved across phyla, we hypothesize that exposure to transition metals will affect the transcription of an evolutionarily conserved set of genes and regulatory pathways. We have completed toxicological and microarray studies of cadmium exposures in yeast, C. elegans, mice, and zebrafish. Further, genome-wide microarray and deletion analysis of yeast exposed to six additional transition metals/metalloids; zinc, mercury, copper, silver, chromium and arsenic; was performed. Approximately 25% of the yeast genes show a greater than 2-fold change in their steady-state level of expression. Gene Ontology, GenMAPP, and Cytoscape analyses show that most metals affected the expressions of genes involved in sulfur assimilation, intermediate metabolism, and ribosome structure and function. Genes for responses to various stresses, including oxidative damage process, and biomolecule transporters were also up regulated. The effects of metals on individual deletion yeast strains were examined to identify sensitive strains and potential molecular targets for metals. Gene functions involved in metal/protein sequestering, trafficking, and signal transduction pathways were shown to be important for defending against metal toxicity. Inter-species comparative genomic analysis among yeast, mouse, zebrafish and C. elegans also identified genes encoding ribosomal and intermediate metabolism proteins as the most significantly differentially expressed genes among all species. The results from this study have identified several novel metal-responsive signaling pathways and detoxification proteins; as well as those typically associated with metal exposure. Many of these pathways are evolutionarily conserved.

W 1286 CHEMISTRY AND BIOLOGY OF CHROMIUM CARCINOGENESIS.

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Hexavalent Cr is a potent human carcinogen that requires intracellular reduction to Cr(III) for the induction of DNA damage and other toxic effects. Cellular reduction of Cr(VI) is largely a nonenzymatic process driven by small molecular weight thiols (cysteine, glutathione) and ascorbate. Due to its more than 10-times higher rate of reduction compared to thiols, ascorbate accounts for the bulk of Cr(VI) reduction in cells in vivo. Cr(VI) reduction generates intermediate Cr(V) and Cr(IV) forms whose relative yield and stability are dependent on the type of the reducer and the ratio of the reactants. Cr(V), which is virtually absent in the reactions of Cr(VI) with ascorbate, has long been considered as a major DNA-damaging species. However, recent mechanistic analyses of Cr-DNA binding, mutagenesis and Cr(VI) genotoxicity in cells with normal and ascorbate-depleted levels showed that Cr(V)-skipping reactions were in fact much more mutagenic and genotoxic. Ascorbate was found to act as a highly potentiating agent for chromate genotoxicity when present inside the cell, but it played a protective role outside the cell by reducing Cr(VI) to poorly permeable Cr(III). Replication of Cr-treated plasmids in human cells showed that ternary Cr-DNA adducts were the main form of mutagenic damage induced by Cr(VI) in different reduction reactions. In contrast to a weak blockage of in vitro replication, ternary Cr-DNA adducts were highly genotoxic in human cells. Inhibition of cellular replication, DNA breakage and apoptotic responses in human and mouse cells were found to result from abnormal processing of Cr-DNA adducts by mismatch repair into secondary, more toxic lesions. Repetitive cycles of exposure to Cr(VI) can lead to the outgrowth of resistant cells lacking mismatch repair, which provides a plausible mechanism for the unusually high frequency of microsatellite instability in chromate-associated human lung cancers. Retention of wild-type p53 by chromate-induced cancers may result from the absence of selective pressure to inactivate this proapoptotic protein since it is not required for Cr(VI)-induced apoptosis and clonogenic lethality.

W 1287 INSOLUBLE NICKEL COMPOUNDS INDUCE GENOTOXIC AND EPIGENETIC EVENTS, GLOBAL DISRUPTION OF GENE EXPRESSION, AND MORHOLOGICAL/NEOPLASTIC TRANSFORMATION OF 10T1/2 MOUSE EMBRYO CELLS.

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Inhalation of sulfidic ore Ni refinery dusts correlated with increased respiratory cancer risk. Inhaled Ni subsulfide (Ni3S2) and green nickel oxide (gNiO) induced respiratory cancer in rats. We hypothesized that carcinogenic, insoluble Ni compounds cause genotoxic and epigenetic events, inducing global disruption of gene expression and cell transformation, in C3H/10T1/2 mouse embryo cells. Ni3S2, gNiO, and black NiO particles were phagocytosed into 10T1/2 mouse embryo cells, generating intracellular Ni(+2) ions, which induced chromosome aberrations

and morphological, anchorage-independent, and neoplastic transformation. RAP-PCR differential display showed that 130 genes were differentially expressed between non-transformed and four Ni-transformed 10T1/2 cell lines, including a) higher mRNA/protein levels from and amplification of, the ect-2 gene, encoding a rho GDP/GTP exchange factor; b) higher calnexin mRNA/protein levels; c) higher Wdr 1 stress-inducible gene mRNA levels; and d)loss of mRNA expression from the DRIP/TRAP-80 gene and two novel genes, in Ni-transformed cell lines. We propose that intracellular Ni(+2) ions a) generate ROS, that induce 1) chromosome aberrations/mutation/gene amplification, activating four oncogenes and 2) mutational inactivation of tumor suppressor genes, and b) cause methylation of the promoters of tumor suppressor genes, silencing tumor suppressor (t. s.)genes. These combined genotoxic events chromosome damage and aberrations/gene amplification) and epigenetic events(DNA methylation) events, activating four oncogenes and inactivating four t. s. genes, lead to deregulated expression of 122 genes and induction/maintenance of morphological/neoplastic cell transformation. Supported by grant R01 ES03341/NIEHS and Contracts NiPERA I-IV, to JRL.

W 1288 EPIGENETIC MECHANISMS OF NICKEL ION CARCINOGENESIS BY INHIBITION OF HISTONE DEMETHYLASES (H3K9).

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Water insoluble Nickel compounds are carcinogenic due to the delivery of high concentrations of Ni ions into cells by phagocytosis and dissolution. The major target of Ni ions in cells is the ascorbic acid, oxoglutarate dependent dioxygenase enzymes. Examples of these enzymes include the HIF-1 prolyl hydroxylase, the Alk B DNA repair enzymes and the newly discovered H3 demethylases. The reason for the specificity in the inhibition is because all contain loosely bound Fe within a His-His glutamic acid facial triad termed the JMJC domain.. Fe binding to this domain is required for catalytic activity. Ni ions readily displace the Fe from all of these enzymes, resulting in essentially inactive enzyme. Nickel ions were found to induce global DNA methylation and H3K9 methylation by inhibiting the H3K9 histone demethylase of which JMDH2A is an example (demethylates H3K9 mono and di-me). Preincubation of recombinant JMDH2A with 5uM Ni ions essentially inactivated the enzyme since even 2000uM Fe was not able to displace the Ni ions from the active site of the enzyme. A ratio of Ni ions to enzyme molecule of approximately 1:1 was required for inhibition. Since Ni ions are one of the least toxic metal ions, this ratio was easily achievable at levels of Ni ions that are not toxic to cells. Transfection of flag tagged JMHD2A resulted in a loss of H3K9 dimethylation which was reversed when cells were treated with Ni ions indicating that this enzyme was a target of Ni ions in intact cells. Mock transfection or transfection of an enzyme with an inactivating mutation in the JMJC domain, did not affect H3K9 dimethylation of cellular chromatin.

W 1289 INTERACTIONS AMONG ARSENIC, CADMIUM, AND LEAD IN CAUSING TOXICITY AND CARCINOGENESIS.

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Humans are exposed to single toxic metals and metalloids, and also to mixtures of toxic and carcinogenic metals and metalloids, in occupational and environmental settings. Metals and metalloids to which humans are exposed in occupational and environmental settings include single exposures to arsenic, cadmium, and lead, and also exposures to mixtures of these metals and metalloids. The mechanisms by which specific metals and metalloids, including arsenic, cadmium, and lead, cause toxicity and carcinogenesis in animals and in humans are of increasing scientific interest. Toxic interactions among arsenic, cadmium, and lead at the molecular level are also of increasing scientific interest. Arsenic, cadmium, and lead interact to synergistically induce oxidative damage to proteins. Low doses of arsenic and cadmium also interact to induce oxidative damage to DNA, including increases in the levels of 8-hydroxy-deoxyguanosine in DNA. It is important to quantify and understand the interactions of these mixtures of metals and metalloids at the molecular level to improve human health risk assessment at low dose levels of these metals and metalloids. Supported by EPA STAR Grant #187261.

W 1290 STRATEGIES FOR ASSESSING DEVELOPMENTAL AND REPRODUCTIVE TOXICOLOGY OF BIO-PHARMACEUTICALS.

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The current ICH Guidance (S6 Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals, July 1997) for reproductive performance and developmental toxicity studies describes a case-by-case situation for every new entity. "The need for reproductive/developmental toxicity studies is dependent upon the product, clinical indication and intended patient population. The specific study design and dosing schedule may be modified based on issues related to species specificity, immunogenicity, biological activity, and/or a long elimination half-life." In addition to the lack of specific guidance, development of monoclonal antibodies and other biological modalities for therapy presents some challenges to traditional practices for assessing developmental and reproductive toxicity. The characteristics of target specificity without off-target toxicity attributed to this type of therapy brings into question the rationale and approaches for standard testing protocols, including the choice of nonclinical test species. Of particular concern is the increased emphasis on using nonhuman primates as a primary test species in reproductive studies. Working groups are addressing the scientific and ethical concerns of using nonhuman primates and developing strategies for standard toxicology testing (Chapman K, Pullen N, Graham M, Ragan I. Preclinical safety testing of monoclonal antibodies: the significance of species relevance. Nat Rev Drug Discov. 2007:120-6). A focused discussion of the strategies for testing bio-pharmaceuticals for potential reproductive and developmental toxicity is merited. This workshop will bring together experts in the fields of reproductive and developmental toxicology, biologics, and regulatory agencies to discuss relevant reproductive testing strategies for bio-pharmaceuticals.

W 1291 ISSUES IN DEVELOPMENTAL AND REPRODUCTIVE TESTING OF BIOPHARMACEUTICALS AND COMMUNICATION OF HUMAN RISK.

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The need for reproductive/developmental toxicity studies is the same for biopharmaceuticals as for pharmaceuticals. Studies are conducted based upon the product, clinical indication and intended population. ICH S5 guidance states that in choosing an animal species and strain for reproductive toxicity testing, care should be given to selection of a relevant model. ICH S5 further states that a single species can be sufficient if the species selected is a relevant model and that nonhuman primates are best used when the objective of the study is to characterize a relatively certain reproductive toxicant, rather than to detect a hazard. In all cases, the advantages and disadvantages of species should be considered in relation to the product, the study design and the subsequent interpretation of the results.

ICH S6 guidance states that the specific study design and dosing schedules may also need to be modified based on issues related to species specificity, biological activity, prolonged serum half-life and/or immunogenicity. Due to these unique product attributes of biopharmaceuticals a number of approaches have been justified on a "case-by-case" basis including: use of non-traditional species, use of homologous test articles, or development of transgenic mouse models. Unfortunately, for sponsors, the wide selection of approaches results in a greater uncertainty at predicting regulatory acceptance of a particular approach.

Since all models have inherent limitations product labels generally contain precautionary statements irrespective of the findings from the animal studies. 'There are, however no adequate and well-controlled studies in pregnant women. Because animal reproduction and developmental studies are not always predictive of human response, 'Product Y' should be used during pregnancy only if clearly needed.' Are there ways to improve the predictive value of the animal studies? What is the value of a class label based upon intended mechanism of action? Can risks be adequately communicated without conducting studies?

W 1292 FDA REGULATORY PERSPECTIVE ON REPRODUCTIVE AND DEVELOPMENTAL TESTING FOR BIOPHARMACEUTICALS.

H. Ghantous. *CDER/DAVP, U.S.-FDA, Silver Spring, MD.*

The need for reproductive and developmental toxicology studies for biopharmaceuticals is dependent upon characteristics of the product as well as the indication and the intended patient population. Reproductive and developmental toxicology studies should be conducted in compliance with the ICH guidance for industry S5A Detection of Toxicity to Reproduction for Medicinal Products, when possible. However, the inherent characteristics of biopharmaceuticals often necessitate flexibility and innovative approaches. Specific study design and dosing schedule may need to be modified based on issues related to species specificity, immunogenicity, biological activity, and/or long elimination half-life. This presentation will address the available guidance documents related to reproductive and developmental toxicology testing including the new draft CDER Biologics Guidance document. The advantages and disadvantages of using non-human primates for reproductive studies will be discussed. In addition, the use of transgenic animals and/or homologue products in reproductive testing of biopharmaceuticals will be considered. Recommendations on the need and design of suitable reproductive toxicology studies are made in the draft Guidance and will be presented.

W 1293 EU REGULATORY PERSPECTIVE ON TOXICITY TESTING FOR REPRODUCTIVE AND DEVELOPMENTAL TOXICITY.

J. W. van der Laan. *Centre for Biological Medicines and Medical Technology, RIVM, Bilthoven, Netherlands.* Sponsor: M. Hurtt.

The administration of recombinant proteins and monoclonal antibodies during pregnancy has always been a matter of special attention. Is it to be expected that the molecules will cross the placenta reaching and affecting the fetus? Testing of recombinant proteins and monoclonal antibodies is associated with a high degree of species specificity leading to the choice of non-human primates as the preferred species. The need for a sufficient number of offspring will lead to the use of a high number of mothers as well. This presentation will provide a review of the number of animals used for this purpose for products reaching the market in Europe from the late 80's until 2002-2003. A large range in number of animals used for testing is related to the number of monkeys per product, which is at least partly due to reproduction toxicity studies. A discussion will be held about the use of homologous products as a way of getting relevant information regarding developmental toxicity, and the use of transgenic mice carrying the required antigens (in case of testing monoclonal antibodies), and the possibility of using another non-rodent mammalian species. The (im) possibility to use minipigs for this purpose needs some attention. A category of biological products requiring attention is the vaccines. New vaccines might be administered during pregnancy accidentally or intentionally. Specific study designs are being considered to establish the safety of such products for potential use during pregnancy.

W 1294 APPROACHES TO TESTING FOR REPRODUCTIVE AND DEVELOPMENTAL TOXICITY: USE OF NONHUMAN PRIMATES.

G. J. Chellman. *Preclinical Services, Charles River Laboratories, Sparks, NV.*

Nonhuman primates are frequently used for reproductive toxicology testing of biological therapeutics, due to considerations related to antigenicity, lack of receptor/target cross reactivity, and/or lack of pharmacologic response in conventional models (rodents, rabbits). Cynomolgus monkeys are most often used, based either on use in the general toxicology program or on the impracticality of other primate species. Depending upon the drug development program, one or more of the following studies may be conducted: fertility (male/female), embryo-fetal development, and/or pre-postnatal development. Nonhuman primates present both advantages and disadvantages in conducting these studies. The selection of which study(s) is needed is determined by the clinical plan, therapeutic area, and regulatory expectations. Each study design is unique and requires scientifically sound, purposeful design. In addition to endpoints that are standard for general toxicity studies, a number of specialized endpoints may also be incorporated into these studies for the mothers and/or infants: e.g., ultrasound, fetal evaluations, multicompartment toxicokinetics, immunological assays, behavioral testing. The addition of these tests needs careful consideration in terms of practicality, cost, and interpretability of data generated (including availability or lack of historical control data). The following concepts will be discussed: optimizing study design(s) relative to limited animal supply and ethical considerations; determining what study(s) are required to support safety assessment and clinical trials; possible use of a single, well-designed study to meet safety and regulatory expectations. The presentation will review the most common reproductive toxicology study designs for nonhuman primates, and provoke thought on how these studies can be optimized in the future.

W 1295 APPROACHES TO TOXICITY TESTING FOR REPRODUCTIVE AND DEVELOPMENTAL TOXICITY: USE OF SURROGATES.

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One alternative to using non-human primates for reproductive toxicity testing (or if the biopharmaceutical does not cross-react with non-human primates) is to use a surrogate (homologous, analogous) molecule. This would be the homologous protein (i.e., the murine protein for use in mice) or, for a monoclonal antibody therapeutic, an antibody that cross-reacts with the rodent target. This allows for the safety testing of the pharmacologic activity of the test agent, but does not allow for testing of the clinical candidate itself. The surrogate molecule should resemble the clinical candidate as much as possible with regards to the production process, range of impurities/contaminants, pharmacokinetics, binding affinity, and pharmacological mechanism. Making and characterizing a second molecule along with the clinical candidate results in the use of a great deal of additional resources, and additional assays must be developed to detect the product and antibodies that might form to the product. However, these efforts can allow for a greater understanding of the potential toxicities of the therapeutic candidate in the more standard toxicology models for assessing reproductive and developmental toxicity. In addition, the use of transgenic or knock-out mice could also be considered as a surrogate for testing the clinical candidate. This presentation will discuss the pros and cons of these approaches and discuss some examples using surrogates for assessing reproductive toxicity.

R 1296 HUMAN HEALTH RISK ASSESSMENT FOR PHARMACEUTICALS IN THE ENVIRONMENT (PIE).

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Numerous studies have documented the presence of low levels of mixtures of emerging contaminants, including human and veterinary medicines, in surface water. These findings are gaining increasing attention. Scientists and the media are questioning the potential impact of these substances on human health and on environmental species. Several issues need to be considered. For example, what are the pathways of entry of human and veterinary medicines to the environment and how can potential exposures be derived from monitoring and modeling data. Significant water reuse in certain regions of the US and the current state of water treatment underscore the importance of valid exposure assessments related to PIE. Preclinical and clinical toxicology, pharmacology and metabolic data generated during the process of drug development and registration can be used to support human health risk assessments. The Federal Interagency PIE Working Group has been formed to identify some of the key definitional issues, data gaps, research priorities and collaborations. Additionally, there are US EPA mandates and regulations that could be applied and critical considerations need to be defined. The session addresses these issues and provides an interactive discussion.

R 1297 DEVELOPMENT OF PHARMACEUTICALS IN THE ENVIRONMENT AS AN ISSUE AND CONSIDERATIONS FOR EXPOSURE ASSESSMENT.

R. T. Williams. *Pharmaceutical Sciences, Pfizer Global Research and Development, Groton, CT.* Sponsor: F. Mastrocco.

Pharmaceuticals have been detected with increasing frequency since the late 1990's in domestic wastewater, surface waters, soils and sediments, and to a lesser extent, drinking water. The number of peer-reviewed papers reporting the detection of pharmaceuticals has increased from less than 5 per year prior to 1996 to over 80 papers in 2006. The average concentration of individual pharmaceuticals reported in surface water from over 38,800 total analyses searching for approximately 300 different pharmaceuticals and metabolites is low, 0.054 parts per billion (ppb, ug/L) or 54 parts per trillion (ppt, ng/L). The average value from over 1200 analyses in drinking water is 0.018 ppb or 18 ppt. The maximum concentration of an individual pharmaceutical reported is 15 ppb (diclofenac and sulfadimethoxine) in surface water and 1.35 ppb (ibuprofen) in drinking water. The source of human pharmaceuticals in the environment is primarily attributed to patient use followed by excretion to wastewater, but unused medicines disposal to wastewater is an exposure pathway receiving worldwide attention. The source of veterinary drugs in the environment is more complex than for human health drugs due to regional and species specific animal husbandry practices. The bioactivity of pharmaceuticals present in the environment has resulted in increasing international attention from the public, as well as from the scientific and regulatory communities, about potential risks to human health and to ecosystems. Two cases of environmental effects, "feminizing" of fish and the decline of vulture populations in SE Asia, have been attributed to estrogen and diclofenac, respectively. No reports of effects to human health from pharmaceuticals in the environment are known, but US FDA and EPA have indicated that the US public has queried the agencies about whether pharmaceuticals in the environment are a human health concern. The adequacy of existing risk assessment science has been challenged, primarily because of the bioactive nature of pharmaceuticals. A comparison of average and maximum surface water concentrations of individual drugs with those needed to elicit a therapeutic or toxic response in mammals or humans has been conducted. Results demonstrate that environmental concentrations are typically several orders of magnitude below the concentrations eliciting therapeutic or toxic responses.

R 1298 TREATMENT AND TREATABILITY OF PHARMACEUTICALS IN WATER TREATMENT PLANTS.

D. Sedlak. *Civil and Environmental Engineering, University of California, Berkeley, CA.* Sponsor: E. Hayes.

In the U.S., approximately 80% of residences and workplaces discharge sewage to sanitary sewers that route to sewage treatment plants with various capabilities to remove chemical contaminants through biodegradation and other means. While

some pharmaceuticals are readily removed during treatment, others pass through treatment plants unchanged. The active pharmaceutical ingredient (API) can also be regenerated from conjugated metabolites of certain drugs by the organisms present in biological treatment systems. Therefore, the discharge of APIs in effluent from sewage treatment plants has the potential to reach drinking water supplies. In addition, combined sewer overflows and leaking sewers can release untreated sewage to water supplies. This pathway can result in the presence in water supplies of APIs that are not efficiently removed in sewage treatment plants. For the purpose of exposure assessment, two scenarios are of particular concern. In the first scenario, which is referred to as unplanned potable water reuse, effluent-dominated surface waters (i.e., surface waters where the flow consists mainly of wastewater effluent) undergo treatment in a conventional drinking water treatment plant. The predominant mechanisms for removal of APIs in surface water treatment plants are oxidation during chemical disinfection and physical removal on activated carbon. Although many APIs will be removed by these mechanisms, not all drinking water treatment plants are equipped with the same processes and it is impossible to assess removal efficiency without knowledge of the treatment plant configuration. In the second scenario, which is referred to as indirect potable water reuse, drinking water is produced from wastewater effluent that is introduced to groundwater or surface water in a well-controlled manner. In one common application, referred to as soil aquifer treatment, additional removal of APIs occurs as wastewater effluent percolates into an aquifer. The groundwater is then abstracted downgradient and used as a water supply with little additional treatment. Advanced technologies are available for enhancing the removal of APIs in drinking water treatment plants. However, the expense of installing such systems is a barrier to implementation.

R 1299 INTERAGENCY PRIORITIES AND COLLABORATION IN ADDRESSING PHARMACEUTICALS IN THE ENVIRONMENT.

H. Zenick. *National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC.*

Under the auspices of the Office of Science Technology and Policy, an interagency Pharmaceuticals in the Environment (PIE) Working Group was established in 2006. The purpose of the PIE Working Group has been to develop a research strategy that 1) defines and prioritizes topic areas and data needs for which the resolution would improve the ability to assess possible human and ecological risk from pharmaceuticals in the environment and 2) recommends areas for federal collaboration to address those priorities. As part of this exercise, an inventory has been established of ongoing PIE activities across the federal government. Interagency discussions have also lead to the development of a novel paradigm on which to base the PIE Strategy. In this paradigm, environmental stewardship (e.g., regulatory or voluntary actions) is the lynchpin with its implementation being informed and guided by the results of research on high priority issues. The latter are defined as, and aligned with, the components of the research diagram that flows from sources → fate and transport → environmental and human exposure → ecological and human health effects. Such an approach creates a dynamic process wherein data delineating the determinants of any of the components of the flow diagram, or the predictive linkages between components, can inform actions that may reduce pharmaceutical contributions to sources (e.g., consumer disposal via flushing) or subsequent source contributions to the environment (e.g., treatment plant discharges). The approach is envisioned to also allow for greater, proactive engagement and interaction between regulatory agencies, state and local governments (e.g., city wastewater treatment managers), key industrial sectors (e.g., pharmaceutical manufacturers, agriculture) and the public. This paradigm can be applied to identify research priorities and areas targeted for cross-agency collaboration.

R 1300 HUMAN HEALTH RISK ASSESSMENT OF PHARMACEUTICALS IN THE ENVIRONMENT: A REGULATORY PERSPECTIVE.

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The U.S. Environmental Protection Agency is charged with initiating research and implementing risk assessment activities to support necessary rule making for ecological and human health protection related to potential environmental exposures. We work within a national framework of protecting human health and the environment using technology and implementing regulations on a watershed basis which is driven by a strong emphasis on sound science, transparency, public information, and partnerships. This framework reflects requirements established by Congress under the Clean Water Act (CWA), the Safe Drinking Water Act (SDWA), and the Food Quality Protection Act (FQPA). Tools within this framework include regulatory authorities such as the Ambient Water Quality Criteria development process (which includes Human Health Criteria), the Contaminant Candidate List selection process, and the Unregulated Contaminant Monitoring Rule. To date, EPA has developed 120 recommended human health criteria and 45 recommended aquatic life criteria for specific chemicals or classes of chemicals. Under the Safe Drinking Water Act and EPA's national drinking water program, the Agency has issued over 200 Public Health Advisories (13 associated with reproductive and developmental endpoints) and established over 85 Maximum Contaminant Level Goals (MCLGs). MCLGs are used in conjunction with information on validated analytical methods, available treatment technologies, and associated costs and benefits to develop enforceable Maximum Contaminant Levels (MCL) or "standards" that apply to approximately 54,000 community water systems that serve over 270 million people across the nation. EPA uses these tools to understand, manage and reduce hazards posed by the occurrence of contaminants such as pharmaceuticals that may have an impact on the health of humans. Other tools include interacting with State governments, developing strong partnerships with other Federal Agencies, and using effective programs such as the Science to Achieve Results (STAR) grants program. All of these tools help ensure that EPA continues to meet the central goal of protecting water quality, human and aquatic health, and assuring safe drinking water. These activities can potentially be used to address the issue of pharmaceuticals in the environment.

R 1301 HAZARD IDENTIFICATION AND HUMAN HEALTH RISK ASSESSMENTS RELATED TO PHARMACEUTICALS IN THE ENVIRONMENT.

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Considerable pre-clinical and clinical toxicology and metabolic data are generated, during the drug development and registration process for human and veterinary medicines. These data can also be used to reduce uncertainty in support of human health risk assessments related to pharmaceuticals in the environment. For example, data from both acute and long term clinical trials, including information on duration of effect, can be used to identify the NOAEL or LOAEL for humans. A comparison of Cmax and/or AUC values from pharmacokinetic/toxicokinetic studies in animals and in humans may allow refinement of the uncertainty associated with interspecies extrapolation. An understanding of metabolic pathways, coupled with comparison of Cmax or AUC values in normal, healthy, adult volunteers vs. patients with a targeted disorder, children, or patients with impaired organ function (e.g. renal, hepatic,) may enhance understanding of inter-individual variability and identification of susceptible individuals/populations. Data from studies of drug interactions in animals and in human trials aid in understanding mechanisms and doses associated with the interactions and can be useful in evaluating possible effects from exposure to mixtures of substances. Available human health risk assessments related to pharmaceuticals in the environment, although limited in number, have generally concluded that there is no significant risk to human health as a result of pharmaceuticals in the environment. Approaches to deal with data issues, e.g. quality of datasets may include the use of additional uncertainty factors, or of de minimis assumptions as well as additional data development.

1302 ARSENIC IMPAIRS PANCREATIC BETA-CELL FUNCTION: INVOLVEMENT OF OXIDATIVE STRESS RESPONSE.

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Human exposure to inorganic arsenic (iAs), a potent environmental oxidative stressor, is associated with Type 2 diabetes (T2D). However, the precise mechanism for the diabetogenic effect of arsenic is still largely undefined. It is clear that defective glucose-stimulated insulin secretion (GSIS) from pancreatic β-cells is an integral element in the progression of T2D. Our recent studies suggested that reactive oxygen species (ROS), such as H2O2, derived from glucose metabolism are important metabolic signaling molecules for GSIS. In contrast, endogenous antioxidants, which rise in response to oxidative stress, have the potential to blunt such glucose-triggered ROS and inhibit GSIS. Here we provide in vivo evidence that iAs exposure through drinking water resulted in pancreatic accumulation of iAs and its methylated metabolites, suggesting pancreatic β-cells may be a target for arsenic toxicity. In isolated mouse islets, a short-term, low concentration of iAs3+ (1 μM) treatment dramatically increased gene expression and protein levels of many antioxidant enzymes including superoxide dismutase, catalase, glutathione peroxidase and

heme oxygenase 1. Following iAs3+-induced elevation of ROS-scavenging activities, a significant decrease in GSIS was observed. Consistent with the findings in islets, exposure of INS-1 (832/13) cells, a glucose-responsive β-cell line, to iAs3+ at a non-toxic concentration (5 μM) for 6 hours resulted in significant nuclear accumulation of transcription factor Nrf2 and induction of its target antioxidant genes. Accordingly, a significantly increased total antioxidant potential and reduced glucose-stimulated intracellular H2O2 accumulation as well as dampened GSIS was observed in the iAs3+-treated cells. Taken together, these data suggest that low-levels of arsenic exposure cause oxidative stress in pancreatic β-cells, leading to activation of antioxidant defense response, scavenging of glucose-derived H2O2 and impaired GSIS.

1303 ABERRANT CYTOKERATIN EXPRESSIONS ASSOCIATED WITH ARSENIC-INDUCED MALIGNANT TRANSFORMATION OF HUMAN KERATINOCYTES.

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Inorganic arsenic is a known human carcinogen. Chronic arsenic exposure results in skin lesions including squamous cell carcinomas. Keratinocytes are thought to be the target cell for arsenic carcinogenesis in skin. Thus, to model arsenic-induced skin carcinogenesis, a human skin keratinocyte cell line (HaCaT) was chronically exposed to 100 nM sodium arsenite, which induced malignant transformation after 28 weeks of exposure. To examine molecular events associated with this transformation, gene expression analysis was performed in cells exposed to arsenic for 4, 10, 20 and 30 weeks. This analysis focused on expression of cytokeratins, several of which are thought to be important markers of the carcinogenic process. Pronounced changes in cytokeratin expression were observed, and approximately half of 24 cytokeratin genes examined showed significant alterations in transcript levels in a time-dependent manner. For example, Cytokeratin 1, an early marker of differentiation associated with hyperkeratosis, was increased between 8- and 34-fold during arsenic transformation. Cytokeratin 13, a biomarker for dermal cancer progression, was increased up to 45-fold during arsenic-induced malignant transformation. This increase in cytokeratin 13 was confirmed at the protein level by Western blot analysis. The stem cell marker, cytokeratin 15, increased up to 7-fold, particularly during the later stages of arsenic exposure, indicating potential emergence of a stem cell-like population with chronic arsenic treatment. Involucrin, a marker protein for keratinocyte differentiation, similarly increased up to 9-fold. These alterations in cytokeratin gene expression often occurred as early as 4 weeks of arsenic exposure, progressively increased with time, and peaked at about 20 weeks of exposure. Thus, chronic arsenic exposure of human keratinocyte HaCaT cells produces malignant transformation together with dramatic and dynamic alterations in cytokeratin expressions that could be important factors in arsenic-induced skin carcinogenesis.

1304 ARSENIC ALTERS FOCAL ADHESION COMPLEXES LEADING TO CHRONIC STIMULATION OF FAK – SRC MEDIATED PATHWAYS.

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Arsenic exposure has been linked tumorigenesis, cardiovascular disease, hypertension, atherosclerosis, and peripheral vascular disease; however, the molecular mechanisms underlying its pathological effects remain elusive. In this study, we investigated arsenic-induced alteration of focal adhesions in normal, primary vascular smooth muscle cells. Integrins respond to cues from the extracellular matrix and initiate intracellular signaling pathways via the assembly of focal adhesions, large multi-protein signaling complexes constructed from a diverse set of molecules including scaffolding proteins, GTPases, kinases, phosphatases, proteases and lipases. Focal adhesions, in part due to their dynamic nature that allows both temporal and spatial regulation of signaling events, are a point of convergence for multiple overlapping pathways including those mediated by GPCRs, RTKs, integrins, and steroid receptors. We demonstrate that exposure to environmentally relevant concentrations of arsenic (50 ppb As3+) can alter focal adhesion complexes and activate integrin-like signaling pathways. Arsenic treatment caused a sustained increase in FAK-src association and activation and the formation of unique signaling complexes (beginning after 3 hours of As3+ exposure and continuing throughout the 12 hour time course). The effects of these alterations were manifested as chronic stimulation of downstream PAK, ERK and JNK pathways. This is a significant finding, as chronic stimulation of these pathways promotes cellular growth, survival, proliferation, migration and gene transcription.

1305 ARSENIC INHIBITS LXR-MEDIATED TRANS-ACTIVATION, BUT NOT TRANS-REPRESSION IN MACROPHAGES.

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Environmental arsenic exposure has been linked to an increased risk of cardiovascular disease in humans. In animal models, arsenic increases atherosclerotic plaque formation and inflammation, although the mechanism by which arsenic induces atherosclerosis is unknown. We have previously shown that arsenic inhibits trans-activation of nuclear receptors that heterodimerize with RXR. Therefore, we hypothesized that arsenic inhibited the trans-activation of LXRs in response to ligands, and that this could explain the pro-atherogenic properties of arsenic exposure. Indeed, arsenic inhibits trans-activation of LXRs in transient transfection assays and endogenous gene expression of the LXR targets ABCA1 and SREBP-1c. Importantly, arsenic treatment is capable of inhibiting ABCA1 and SREBP-1c mRNA expression when administered at low doses (100 nM and 250 nM) over several weeks, an experiment which more closely models the effects of exposure to arsenic as an environmental contaminant. Arsenic treatment did not alter nuclear receptor mRNA and protein expression. In mammalian two-hybrid and co-immunoprecipitation assays, arsenic did not affect the heterodimerization of LXRs with RXR. We assessed protein occupancy at the LXRE sites of both ABCA1 and SREBP-1c promoters using ChIP assays. Arsenic treatment is associated with decreased histone H3 acetylation and increased N-CoR recruitment, although it does not affect occupancy of LXRs or RXR on either promoter. Arsenic, as expected, can induce the expression of inflammatory genes, such as Cox2, iNOS, and IL-8, but is unable to inhibit LXR-mediated trans-repression of these genes. Trans-repression by nuclear receptors has been linked to their SUMOylation. Preliminary data show that arsenic does not alter ligand-mediated SUMOylation of LXRs. Indeed, arsenic itself directly SUMOylates LXRs. These data suggest that arsenic may increase atherosclerotic lesion formation by inhibiting LXR trans-activation, potentially through their SUMOylation, but is unable to inhibit their trans-repressive function.

1306 γ-GLUTAMYL TRANSFERASE (GGT1) EXPRESSION LEVEL IS ASSOCIATED WITH ARSENIC RESISTANCE IN NORMAL HUMAN LYMPHOBLASTS: A MICROARRAY STUDY.

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Exposure to arsenic in drinking water is associated with increased skin, lung and bladder tumors, as well as other diseases. 14 lymphoblast lines derived from normal unexposed caucasian females (Coriell Institute) were assayed for sensitivity to growth inhibition by sodium arsenite. Wide variability in sensitivity to arsenite was found. Microarray analysis was used to compare basal gene expression profiles between two arsenite-resistant and two arsenite-sensitive lymphoblast lines. After normalization, gene expression levels in each of the two sensitive cell lines was divided by the expression levels of each of the two resistant cell lines, resulting in four ratios (S1/R1, S2/R1, S1/R2, S2/R2) for each oligonucleotide. A gene was deemed as differentially expressed if either all four ratios exceeded 2, or all four ratios were less than 0.5. 40 genes were found to be differentially expressed. Among these was GGT1, which showed higher expression levels in both resistant cell lines. These results were confirmed with RT-PCR analysis. Reduction of GGT1 expression levels in the two arsenite resistant lines with GGT1-specific siRNA resulted in increased sensitivity to arsenite. Arsenite is extruded from cells by some members of the multi-drug-associated protein family (MRP) as arsenic-triglutathione [$As(GS)_3$], which becomes arsenite + 3GSH outside the cell. Once extruded, GSH cannot re-enter cells. GGT1 is localized on the outer surface of the cell membrane. It cleaves the gamma-glutamyl bond of GSH to produce cysteinylglycine (Cys-Gly). Cys-Gly hydrolase then cleaves the dipeptide. The resulting amino acids can now enter the cell to regenerate GSH. In this way, GGT activity protects cells from GSH depletion and oxidant-induced cytotoxicity. In conclusion, we have demonstrated that using expression microarray analysis of human cells with varying sensitivities to toxicants may be a useful method for identifying possible Biomarkers of sensitivity.

1307 POTENTIAL GENERATION OF CANCER STEM CELLS DURING ARSENIC-INDUCED MALIGNANT TRANSFORMATION OF HUMAN UROGENITAL PROGENITOR CELLS.

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Inorganic arsenic is a human carcinogen linked to cancers of the urogenital system (UGS) including the urinary bladder and the prostate. Our recent data from mice suggest that, during fetal exposure, arsenic may target an undifferentiated,

stem/progenitor cell population to induce tumor formation, potentially through the production of cancer stem cells (CSCs). We examined the effects of chronic, low-level in vitro arsenic (5 μM) exposure on the non-tumorigenic human UGS stem/progenitor cell line, WPE-stem. WPE-stem cells form floating clusters of viable cells, termed "prostaspheres." Prior work shows that stem/progenitor cells are often present in these floating spheres and spheres isolated from cancer cell lines can contain chemoresistant and aggressive CSCs. Arsenic induced malignant transformation in WPE-stem cells in only 18 weeks based on various criteria. Upon injection of potentially transformed cells into Nude mice, highly pleomorphic, invasive and metastatic carcinosarcomas developed in as little as 3 weeks, suggesting a stem-like cell origin. Further, WPE-stem transformants formed floating spheres at a rate >2.3-fold above control. In addition, MMP-9 activity, a consistent marker for arsenic transformation, was >670% higher in the arsenic-treated cell spheres than in control (adherent and prostaspheres) and in the arsenic-treated adherent cells. Thus, it appears that the true malignant cells, conceivably CSCs, reside in the spheres formed in the arsenic-treated cells. Time course analysis of differentiation/stem cell markers (p63, cytokeratins, etc.) suggests that arsenic may target a stem/progenitor cell population causing aberrant differentiation in normal UGS cells, followed by de-differentiation back to a more stem-like but malignant state, potentially leading to the development of CSCs and eventually cancers. These studies help to define early events in arsenic carcinogenesis, and provide insight into molecular mechanisms involved in CSC development.

1308 CHRONIC LOW-DOSE ARSENIC EXPOSURE ALTERS KEY REGULATORS OF INNATE IMMUNE RESPONSE *IN VIVO*.

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Arsenic (As) exposure is a significant worldwide environmental health concern. Through mechanisms that remain unclear, chronic exposure to As in the drinking water has been associated with many diseases, including cancer, cardiovascular disease and diabetes. In this study, Affymetrix whole genome 430 2.0 microarrays were used to assess global gene expression changes in the lungs of C57BL/6J mice (9 wk, n=5 per treatment group) housed on a casein-based AIN-76A diet and exposed to environmentally relevant concentrations (10 and 100 ppb) of As in drinking water for 5 wk. Rank product analysis (p value < 0.05) was implemented comparing As exposures and control, revealing significant alterations in the expression of many genes involved in innate immunity. The innate immune response is the body's first mechanism for defense against pathogens and is capable of inducing inflammation and, if necessary, the adaptive immune response. Interleukin 1B (IL1B), Interleukin 1 Receptor 2, a number of Toll-like receptors and several cytokines/cytokine receptors were altered in the As-exposed mice. Real-time RT-PCR and Western blot analysis confirmed the altered regulation of a number of these genes at the mRNA and protein levels. Interestingly, a significant decrease in IL1B mRNA expression was observed in the As exposed mice, but Western blot analysis revealed that the protein levels of IL1B were significantly increased with chronic As exposures as low as 10 ppb, suggesting potential compensatory regulation between the mRNA and protein, which is known to be extremely complex. These findings indicate that chronic low-dose As exposure can elicit effects on the regulation of innate immunity which may contribute to altered disease risk. (NIH-NIEHS SBRP P42 ES007373 P2)

1309 INTERPLAY BETWEEN CELLULAR METHYL METABOLISM AND ADAPTIVE EFFLUX DURING CHRONIC ARSENIC EXPOSURE IN HUMAN CELLS.

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Arsenic, a human carcinogen that may target the prostate, is enzymatically methylated in many cells using S-adenosylmethionine (SAM) as the methyl donor. After chronic low-level arsenic exposure, the human prostate cell line RWPE-1, which cannot methylate arsenic, acquires a malignant phenotype with DNA hypomethylation, indicative of disrupted methyl metabolism, as well as arsenic adaptation featuring increased glutathione production and arsenic efflux, likely as a glutathione complex. Thus, the interplay between methyl and glutathione metabolism during arsenic adaptation was studied. Cells exposed to low-level inorganic arsenic (5 μM) showed progressive increases in LC_{50} that were > 3-fold above control by 16 weeks. During arsenic adaptation, SAM levels were reduced up to 35%. Early decreases in expression of methionine adenosyltransferase 2A (converts methionine to SAM) and a time-dependent increase in S-adenosylhomocysteine (SAH) hydrolase (converts SAH to homocysteine; 250%) occurred, indicating over-production of homocysteine (Hcy) but reduced conversion to methionine and SAM. Indeed, a marked increase in Hcy levels (180%) occurred. Thus, it appears SAM recycling is reduced in favor of over-production of Hcy during arsenic adaptation. The transsulfuration pathway, which produces cysteine from Hcy, was also upregulated through increased expression of cystathionase. Glutathione production genes, such as glutamate cysteine-ligase (800%) were over-expressed, resulting in increased glutathione levels (500%) during arsenic adaptation. Expression of the ABCC1 transporter, which effluxes arsenic as a triglutathione complex, was also markedly increased. Thus, arsenic adaptation is a dynamic process that involves decreased SAM recycling and Hcy accumulation. Hcy is used in the transsulfuration pathway to increase glutathione production and enhance arsenic efflux resulting in a better short-term survival but ultimately disrupting the cellular methyl homeostasis. These events appear to occur regardless of the ability to methylate arsenic.

1310 THE IMPACT OF GLYCOSYLATION ON IGE ANTIBODY RESPONSES.

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Human native and recombinant forms of the iron binding protein lactoferrin (nLF and rLF) are available which have identical amino acid sequences but differential glycosylation patterns. We have characterized IgG and IgE antibody responses induced in BALB/c strain mice by these proteins. Mice were immunized with various concentrations (w/v) of nLF or rLF alone or in combination by intraperitoneal injection on day 0 and 7 and exsanguinated on day 14. Control mice were immunized with the irrelevant allergen ovalbumin alone or in combination with rLF. Sera were analyzed for protein specific IgG by enzyme-linked immunosorbant assay and for protein-specific IgE by homologous passive cutaneous anaphylaxis assay (PCA). Exposure to nLF (0.2% to 1%) alone resulted in vigorous IgG and IgE responses, even at the lowest dose tested. In contrast, administration of rLF (1% to 5%) alone resulted in less vigorous IgG responses and no detectable IgE antibody, except at the highest dose tested where a minority of animals displayed low titer IgE antibody. Immunisation with a combination of rLF (1%) and 0.2% nLF inhibited completely the specific IgE response provoked by nLF. However, rLF (1%) was without effect on IgE antibody induced by the unrelated allergen ovalbumin (0.2%) using an identical immunization regimen. Failure of rLF to stimulate vigorous IgE antibody was not due to loss of IgE binding epitopes, as similar antibody titers were observed regardless of the form of LF used as challenge substrate in the PCA. The ability of rLF to inhibit specifically IgE antibody responses to nLF suggests that the differential glycosylation patterns influence the induction phase and are probably important for antigen processing and/or presentation.

1311 LIMITING ALLERGEN EXPOSURE DURING DEVELOPMENT: DIFFERENTIAL EFFECT ON ASTHMA SYMPTOMS IN A MOUSE MODEL.

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Reducing exposure to allergens improves asthma control and reduces characteristic symptoms of asthma such as lung inflammation and airway hyperresponsiveness (AHR). We hypothesized that a greater reduction in allergen exposure was necessary to limit asthma symptoms in young animals compared to adults, regardless of sex. Female and male Balb/c mice were sensitized on postnatal day 19 (Y/Y) or 82 (A/A) with varying doses of ovalbumin (OVA; 0.005-50 mg/kg) or saline with alum. On days 14, 15, and 16 after sensitization, animals were challenged intranasally with 5 mg/kg OVA. Eosinophil infiltration, AHR and OVA-specific IgE were determined on day 17 after sensitization. Sex differences in eosinophilia were evident since females, whether Y/Y or A/A, exhibited statistically significant eosinophilia at 10 times lower OVA doses than males. Considering age dependence, Y/Y and A/A animals of the same sex exhibited statistically significant increases in eosinophilia over the same range of OVA sensitizing doses. However, the maximum eosinophilia was significantly greater in A/A compared to Y/Y, consistent with greater OVA-specific IgE in A/A than Y/Y animals. After OVA sensitization and challenge, AHR was determined using either unrestrained barometric plethysmography (PenH) or the forced oscillation technique (airway resistance). Using PenH and aerosol methacholine, young females exhibited AHR at 10-100 times lower sensitizing OVA doses than young males or adult animals. Using changes in airway resistance to intravenous methacholine, AHR was evident in A/A animals sensitized and challenged with OVA, but not in Y/Y animals as seen with PenH. The results of these studies clearly indicate that the effectiveness of limiting allergen exposure will differ with age, sex and asthma endpoint being measured. (Support: Department of Defense CDMRP; DAMD 17-02-1-0191).

1312 LOCAL ANTIBODY CLASS SWITCHING IN THE NASAL MUCOSA OF MICE WITH TDI RHINITIS.

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The role of antibodies in the etiology of occupational rhinitis and asthma caused by diisocyanate exposure is controversial. Serum levels of diisocyanate-specific IgE and IgG antibodies are detectable in approximately 30% and 80% of exposed workers, respectively. Importantly, specific IgE antibodies have high specificity for disease whereas specific IgG is present in the serum of exposed workers without disease indicating that these antibodies may be markers of exposure. The low detection prevalence of diisocyanate specific antibodies may be due to methodological limitations or low production and utilization of these antibodies at sites of active inflammation. Therefore, we used a previously developed murine model of TDI rhinitis to test the hypothesis that inhalation of diisocyanate vapor results in antibody class switching at the site of exposure, the nasal mucosa. Antibody class switching involves expression of germline transcripts and activation-induced cytidine deaminase (AICD) leading to recombination and production of a mature immunoglobulin transcripts. We employed PCR-based assays to detect germline and mature immunoglobulin transcripts, AICD and IL-4, a cytokine known to drive the process. Mice with TDI rhinitis showed increased expression of IL-4 and AICD. Strong upregulation of mature transcripts for IgG1 and its corresponding germline transcript were observed in mice with TDI rhinitis. In addition, germline and mature transcripts for IgE were detected in some of the TDI treated mice but not in the control mice. Mature and germline transcripts for IgA were detected in approximately 50% of the control mice and were upregulated in all of mice exposed to TDI. Overall, these results provide evidence that antibody class switching occurs in the nasal mucosa following inhalation of TDI. Determining the specificity of local antibodies and their role in respiratory allergies caused by diisocyanates will be important in protecting the health of potentially exposed workers.

1313 EVALUATION OF IRRITANCY AND THE SENSITIZATION POTENTIAL OF METAL WORKING FLUIDS AND METAL WORKING FLUID COMPONENTS.

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Metalworking fluids (MWFs) are used to reduce the heat and friction associated with industrial machining and grinding operations. There are approximately 1.2 million workers potentially exposed to MWFs. Irritancy and sensitization potential of 9 National Toxicology Program nominated MWFs (TRIM 229, TRIM VX, TRIM SC210, CIMTECH 310, CIMPERIAL 1070, CIMSTAR 3800, SYNTILO 1023, SUPEREDGE 6768, and CLEAREDGE 6584) were examined in a combined local lymph node assay (LLNA). BALB/c mice were dermally exposed to each MWF at concentrations up to 50%. Significant irritation was observed after dermal exposure to all MWFs except CIMTECH 310 and SYNTILO 1023. Of the 9 MWF tested, TRIM VX yielded the highest sensitization potential with an EC3 value of 6.9%. Chemical components of TRIM VX identified using HPLC, were screened for sensitization potential using QSAR modeling and the LLNA. TOPKAT predicted Triethanolamine (TEA) and Triton-X as sensitizers while DEREK for Windows only predicted 4-Chloro-3-methylphenol (CMP). When tested in the LLNA only CMP (EC3= 28%) and OA (EC3=30%) were identified as sensitizers. All TRIM VX components tested resulted in a significant irritation response. In a further attempt to explain the low EC3 value for TRIM VX, combinations of the components were analyzed in the LLNA. Dermal application of mixtures of CMP, OA, TEA, and alpha-terpineol (TMP) resulted in an EC3 value for the mixture of 3.9%. Mixtures of CMP and OA resulted in an EC3 value of 5.9% while mixtures of CMP, TMP, and OA resulted in an EC3 value of 5.8%. These results suggest that the mixture may be responsible for the magnitude of sensitization potential of TRIM VX as compared to the sensitization potential of the individual components. This raises the question if testing individual components of a mixture or manufactured product will accurately predict the sensitization potential of final products.

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1314 EVALUATION OF A GENOMIC APPROACH TO THE LOCAL LYMPH NODE ASSAY (LLNA).

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Genomic technologies have the potential to enhance and complement existing toxicology endpoints. The present study was conducted to compare the sensitivity of genomic responses to the traditional LLNA endpoint of lymph node cell proliferation and to evaluate the responses for their ability to provide insights into mechanisms of action. Female BALB/c mice (9/group) were dosed on the dorsum of each ear on study days 1, 2 and 3 with vehicle, 0.1, 1 or 10% trimellitic anhydride (TMA), a strong sensitizer. On day 6, four animals from each dose group were injected with tritiated thymidine (3HTdR) and 5 hours later the auricular lymph nodes (ALN) were removed and processed for 3HTdR incorporation. The remaining 5 mice/group were sacrificed on day 6 and the ALN were harvested for gene expression analysis. Additional mice dosed with either vehicle or 10% TMA and sacrificed on day 3 or 10, were also included to examine temporal effects on gene expression. Analysis of 3HTdR incorporation revealed TMA induced stimulation indices of 2.8, 22.9 and 61.0 relative to vehicle with an EC3 of 0.11%. Examination of gene expression responses identified 9, 833, and 2122 differentially expressed genes relative to vehicle for the 0.1, 1 and 10% TMA dose groups, respectively, while no changes were identified in an untreated control group. Calculation of EC3 values for differentially expressed genes did not identify a response that was more sensitive than the 3HTdR value. However, a number of genes displayed comparable sensitivity including IL4, Akr1c18, Gzmb and IL21 which exhibited EC3 values of 0.12, 0.15, 0.27 and 0.39%, respectively. Furthermore, these and other responses could be mechanistically linked to sensitization/allergy and Th2 cellular responses. Collectively these results suggest gene expression responses may provide mechanistic surrogates for the detection of sensitizers. Furthermore, a comprehensive examination of this approach using a diverse spectrum of chemicals could aid in differentiating contact and respiratory sensitizers.

1315 HEME-OXYGENASE 1 AND NADPH QUINONE OXIDOREDUCTASE 1: NEW TARGET GENES TO PREDICT THE SENSITIZING POTENTIAL OF CHEMICALS ?

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Contact sensitizers can mimick danger signals such as LPS or cytokines by inducing the maturation of dendritic cells. In vitro studies has previously shown that nickel and dinitrochlorobenzene (DNCB) induced phenotypical changes in human dendritic cells generated from CD34+ hematopoïetic progenitors (CD34-DC). The purpose of this study was to identify new activation markers in response to contact sensitizers for developping in vitro methods to predict the sensitizing potential of chemicals. Recent in vitro studies have demonstrated that contact sensitizers could induce an oxidative stress in dendritic cells (DC). In this work, we focused our investigations on the Nrf2/Keap1 pathway, a highly sensitive pathway in response to oxidative stress. Two models known to respond to contact senzitizers have been used: the U937 monocytic cell line and CD34-DC. Our results showed that the expression of target genes of Nrf2 such as HMOX1 (heme-oxygenase 1) and NQO1 (NADPH quinone oxydoreductase 1) are up-regulated in a dose-dependent manner in response to NiSO4 and DNCB both in U937 cells and CD34-DC suggesting that these molecules induced an oxidative stress. In U937, both HMOX1 and NQO1 expression are induced by nickel and DNCB 8 hours after treatment. In CD34-DC, 14 hours are necessary to induce these two genes. We are currently investigating the potential of other contact sensitizers belonging to different classes of chemical such as formaldehyde, para-phenylenediamine and hexylcinnamaldehyde to induce HMOX1 and NQO1 gene expressions.

1316 ZINC DIETHYLDITHIOCARBAMATE ALLERGENICITY: POTENTIAL HAPTENATION MECHANISMS.

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Zinc diethyldithiocarbamate (ZDEC) and its disulfide, tetraethylthiuram disulfide (TETD) are rubber accelerant contact allergens that cross-react in some individuals. Rubber gloves are often worn while using oxidizing disinfectants. The present study explored potential protein haptenation mechanisms of ZDEC and its oxidation products. Oxidation of ZDEC by bleach, iodine or hydrogen peroxide resulted in production of TETD, tetraethylthiocarbamoyl disulfide (TETCD) and tetraethyldicarbamoyl disulfide (TEDCD). Cysteine, glutathione and albumin thiols reduced TETD with subsequent mixed disulfide formation suggesting a potential route of protein haptenation. Chelation of the copper ion bound on the active site of the monomeric Cu/Zn superoxide dismutase (SOD) by diethyldithiocarbamate was observed when ZDEC was reacted with SOD. The specificity of the ZDEC-Cu(II) reaction and lack of ZDEC reactivity with reduced Cys6 and Cys111 on SOD suggested formation of the DEC-Cu(II) co-ordinate bond as another haptenation mechanism. The murine local lymph node assay (LLNA) was used to evaluate sensitization potential of ZDEC, Cobalt-DEC (CoDEC), TETD and TEDCD. The ranking of the sensitization potency was ZDEC ≥ TEDCD >

TETD >> CoDEC (no sensitization achieved) with EC3 values 1.01, 1.70, 5.42 and >7.5 respectively. CoDEC does not bind to Cu-proteins or form mixed disulfides with free thiols demonstrating the importance of these properties in ZDEC sensitization. Paradoxically, oxidative substitution of S by O on the thiocarbamates of TETD (= TEDCD) was allergenic, but was not subject to haptenation through mixed disulfides or metal chelation. It is concluded that ZDEC may haptenize proteins through (1) chelation of metals on metalloproteins, (2) TETD mediated formation of mixed disulfides, and (3) possibly through nucleophilic addition onto an TEDCD electrophilic carbamoyl group.
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1317 ATROPINE BUT NOT METHYLATROPINE CORRECTS PARAOXON-INDUCED RESPIRATORY DISTURBANCES.

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Objective: The organophosphate cholinesterase inhibitor paraoxon (PO) induces respiratory toxicity but the mechanism is still unclear. We previously showed that atropine (A) induced a complete correction of the respiratory toxic effects. The aim of this study was to compare the effects of A and methylatropine (MeA, a quaternary analog with limited access to the central nervous system) to clarify the peripheral or central origin of the respiratory toxicity. Methods: Male Sprague-Dawley rats were given PO (0.215 mg.Kg-1, sc) and treated with A (base: 10 mg.Kg-1, sc) or equimolar MeA (base: 5.42, 54.2, and 542 mg.Kg-1, sc) 30 min after PO. Respiratory function was assessed using whole body plethysmography and central temperature using infra-red telemetry. Results are expressed as mean +/- SEM. Statistical analysis used non-parametric ANOVA tests with $p< 0.05$. Results: PO induced a significant decrease in temperature 30 min after injection lasting 90 min. This effect was partially corrected by A, but not by MeA. PO induced an increase in expiratory time and tidal volume, and a decrease in respiratory rate. Comparing the effects of equimolar doses, A (10 mg.Kg-1) completely reversed the PO-induced respiratory alterations while MeA (5.42 mg.Kg-1) had no significant effect. Using 10- (54.2 mg.Kg-1) and 100-fold (542 mg.Kg-1) higher dosages, MeA still had no significant effect on respiratory changes. Discussion: In contrast with A which can cross the blood-brain barrier and modify both peripheral and central muscarinic effects, MeA does not cross the blood-brain barrier. PO poisoning induced hypothermia was partially corrected by A but not by MeA. A completely reversed PO-induced respiratory disturbances, while MeA had no significant effect at dosages up to 100-fold higher than A. We conclude that respiratory toxicity induced by PO is primarily mediated by disrupted muscarinic signaling in the central nervous system.

1318 TISSUE CHOLINESTERASE ACTIVITIES DO NOT EXPLAIN REBUND OF PAROXON-INDUCED RESPIRATORY TOXICITY AFTER PRALIDOXIME IN RATS.

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Objective: We previously showed that a single dose of pralidoxime (PRX) methylsulfate induced a complete but transient correction of the effects of paraoxon (PO) on respiration at rest contrasting with a sustained reactivation of whole blood cholinesterase activity (ChEA). However, we did not measure the ChEAs in tissues at the time when the PO-induced respiratory effects reappeared. In the present study, we studied the effect of PRX on reactivation of tissue CHeAs. Methods: Sprague-Dawley male rats were poisoned using PO (0.215 mg.Kg-1 i.e. 50% LD50) administered subcutaneously. PO-poisoned rats were treated with either saline or PX (base: 50 mg.Kg-1, IM) 30 min post injection of PO and sacrificed 60 min after. CHeAs were measured by radiometric assay in whole blood (WBChEA), diaphragm, lungs, brainstem, thigh muscle, and brain frontal area. Results are expressed as mean +/- SEM and compared using non-parametric tests. Results: compared to the control group, PO poisoning induced a significant inhibition ($p< 0.01$) in WBChEA (residual activity: 42 %) and in all tissues (residual activities ranging from 58 to 74 %) except for the frontal area. Ninety minutes after PRX administration, a complete reactivation of WBChEA was observed. PRX also induced a complete reactivation of CheA in the brainstem and a reactivation resulting in more than 75% of residual AChEAs in the other tissues. Discussion: in this model, WBChEA inhibition was not well correlated to CheA inhibition in various tissues. Within the different areas of the brain, PO effects, assessed by CheA inhibition, were major in the brainstem but completely reversed by PX. PX also significantly improved the ChEA in diaphragm. Our data suggest that the rebound in PO toxicity on the respiratory system at 60 min after PO administration involves other mediators than only the cholinergic system.

1319 ALL ORGANOPHOSPHATES ARE NOT THE SAME: DEVELOPMENTAL EXPOSURES TO DIAZINON OR PARATHION PRODUCE DIFFERENT CELL SIGNALING OUTCOMES.

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Although organophosphates (OPs) share the ability to inhibit cholinesterase (ChE), their deleterious actions on development reflect other mechanisms operating at doses below the threshold for inhibition. We evaluated exposures straddling the threshold for barely detectable ChE inhibition, comparing the effects of diazinon (0.5 or 2 mg/kg/day) and parathion (0.1 and 0.2 mg/kg/day) administered to rats on postnatal days 1-4. We assessed the long-term effects on cardiac cell signaling mediated by adenylyl cyclase (AC) in adolescence (postnatal day PN30), early adulthood (PN60) and full adulthood (PN100). In adolescence, diazinon evoked global upregulation of AC activity in males, including the responses to stimulatory G-protein-coupled receptors (β-adrenergic receptors, glucagon receptors). Although this pattern waned by young adulthood, the elevations in receptor-mediated AC responses reemerged in full adulthood. In contrast, females showed initial suppression of receptor-mediated responses that disappeared by young adulthood and were not evident in full adulthood. Parathion displayed very different effects, with an overall decrease in AC in males at adolescence that was no longer evident in full adulthood; females showed an increase in AC at adolescence, the opposite effect from that seen with diazinon. Our results support the view that OPs target cell signaling cascades at doses below the threshold for ChE inhibition and therefore can differ in their developmental outcomes. These effects are distinctly sex-selective. Finally, because cell signaling alterations extend to peripheral tissues, the adverse effects of OPs are likely to have an impact on cardiovascular and metabolic function. Support: NIH ES10356 and ES07031.

1320 CRITICAL MOLECULAR PATHWAYS OF NEUROGENESIS IN CHLORPYRIFOS RESPONSE.

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Exposure to pesticides such as chlorpyrifos (CP) during critical "windows of susceptibility" has been hypothesized to alter behavior and development of the central nervous system (CNS) by interfering with the regulatory pathways that control the production and selective cell loss of neurons. Genomics, through its ability to simultaneously monitor thousands of genes at same time, has proved to be an efficient method for identifying gene pathways involved in the process of normal and abnormal neurodevelopment. Using Affymatrix mouse whole genome arrays, we applied this technique to characterize gene expression alterations due to CP exposure and its impact on CNS development. Pregnant mice (GD6) were administered doses of 0, 2, 4,10, 12, 15 mg/kg/d CP. Total RNA was extracted from maternal brain and fetal tissues and samples were prepared for hybridization to Affymetrix mouse whole genome oligonucleotide arrays. To establish the associations between the treatment and the affected gene ontology (GO) term and pathways, we used DAVID 2006 and the GenMAPP program. We also applied our recently developed system-based GO-Quant approach to quantitatively evaluate the dose dependent response in functional gene pathway. We found CP exposure resulted in significant gene expression changes, 322 and 1372 genes in maternal and fetal brains respectively (ANOVA, $p\leq 0.005$). Hierarchical clustering analysis demonstrated distinct differential gene expression patterns between maternal brain and fetus brain tissue. In addition, gene ontology (GO) and pathway analysis showed that CP exposure effected multiple functional targets. Targets in maternal tissue included cell communication, protein modification, nervous system development, while fetal tissue targets included RNA metabolism and regulation of cell growth. These results highlight the differential response to CP between the tissues and further our understanding of the critical molecular pathways of neurogenesis disrupted by CP gestation exposures. (NIEHS/NIH 1-U10-ES-11287-01 and 2-P01-ES009601 and EPA RD-8370901-0)

1321 EFFECTS OF ATRAZINE ON NEUROENDOCRINE FUNCTION IN WISTAR RATS.

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Previous work has shown that exposure of female Sprague-Dawley rats to high doses of atrazine causes a suppression of the luteinizing hormone (LH) surge and the disruption of the estrous cycle, resulting in prolonged periods of exposure to endogenous estrogen followed by the earlier appearance of mammary tumors. The

purpose of this study was to investigate the effects of atrazine on the neuronal control of gonadotropin releasing hormone (GnRH) release. The secretion of GnRH into the hypophyseal portal blood is characterized by two patterns: 1) an episodic pattern whereby GnRH and LH are released intermittently during the day and 2) a preovulatory surge of GnRH and LH that in rodents is timed to the light cycle. In this study, we characterized the effects of ATRA on GnRH neuronal activation by measuring two indicators: pituitary LH release and cFOS expression in GnRH neurons. In Experiment 1, ovariectomized Wistar rats were administered ATRA (0, 50, 100, 200 mg/kg/day) by gavage for four days. On the 4th day, blood was collected every 5 minutes for 3 hours to determine episodic LH release. ATRA decreased LH pulse frequency and increase pulse period and amplitude. In experiment 2, ovariectomized Wistar rats, engineered to express a green flourescent protein in their GnRH neurons, were administered ATRA for 4 days as in Experiment 1 and treated with estrogen and progesterone to induce a LH surge. Plasma LH was measured in one sub-group and a second sub-group was sacrificed to determine the percentage of GnRH neurons expressing cFOS, a protein normally expressed in GnRH neurons during the preovulatory surge of LH. A significant reduction in the percent of GnRH-cells immunoreactive for cFOS was observed in the 100 and the 200 ATRA-treated groups. These results suggest that ATRA blocked GnRH neuronal activation at a time when the LH surge is suppressed.
Supported by Syngenta Crop Protection Inc.

1322 PROTEOMIC ANALYSIS OF DIAMINOCHLOROTRIAZINE (DACT) ADDUCTS IN WISTER RAT PITUITARY GLANDS AND LβT2 RAT PITUITARY CELLS.

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Atrazine (ATRA) is the most commonly applied herbicide in the United States and is frequently detected in drinking water at significant levels. Following oral exposure, ATRA metabolism yields diaminochlorotriazine (DACT), an electrophilic molecule that has been shown to form covalent protein adducts. This research was designed to identify ATRA-induced protein adducts formed in the pituitary gland of ATRA exposed rats and in DACT exposed LβT2 rat pituitary cells. Immunohistochemistry showed diffuse cytoplasmic and nuclear staining in both pituitary sections and LβT2 cells indicating the formation of DACT protein adducts. Protein targets from both rat pituitaries and LβT2 cell culture were identified following 2-dimensional electrophoresis, immunodetection, and MALDI-TOF mass spectrometry analysis. Western blots from both exposed rats and LβT2 cells revealed over 30 DACT-modified spots that were not present in control animals. Protein spots were matched to concurrently run 2-DE gels stained with Sypro Ruby, excised, and in-gel digested with trypsin. Mass spectrometry analysis of digest peptides resulted in the identification of 19 spots and 8 unique proteins in the rats and 21 spots and 19 unique proteins in LβT2 cells. The identified proteins present in both sample types included proteasome activator complex subunit 1, ubiquitin carboxyl-terminal hydrolase isozyme L1, tropomyosin, ERp57, and RNA binding proteins. Each of these proteins contains active site or solvent exposed cysteine residues, making them viable targets for covalent modification by DACT.

1323 HEPATIC TOXICOGENOMIC RESPONSES TO TRIAZOLE ANTIFUNGALS CONSERVED BETWEEN RODENTS AND HUMANS.

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The triazole antifungals myclobutanil (MYC), propiconazole (PPZ) and triadimefon (TDF) all disrupt steroid hormone homeostasis and cause varying degrees of hepatic toxicity. To identify biological pathways consistently activated across various study designs, gene expression profiling was conducted on livers from rats following acute, repeated dose, or prenatal to adult exposures. To explore conservation of responses across species, gene expression from these rat in vivo studies were also compared to in vitro data from rat and human primary hepatocytes exposed to MYC, PPZ, or TDF. Pathway and gene level analyses across time of exposure, dose, and species identified patterns of expression common to all three triazoles, which were also conserved between rodents and humans. Pathways affected included androgen and estrogen metabolism, xenobiotic metabolism signaling through CAR and PXR, and CYP mediated metabolism. Many of the differentially expressed genes are regulated by the nuclear receptors CAR, PPARalpha and PXR, including ABC transporter genes (Abcb1 and MDR1), genes significant to xenobiotic, fatty acid, sterol and steroid metabolism (Cyp2b2 and CYP2B6; Cyp3a1 and CYP3A4; Cyp4a22 and CYP4A11) and xenobiotic clearance (Ugt1a1 and UGT1A1). Modulation of hepatic sterol and steroid metabolism is a plausible mechanism for triazole induced increases in serum testosterone. The gene expression changes caused by all three triazoles appear to focus on pathways regulating lipid and steroid homeostasis, identifying potential common mechanisms that are conserved in rodent and human livers. [This work was reviewed by EPA and approved for publication but does not necessarily reflect official Agency policy.]

1324 TRANSCRIPTIONAL RESPONSES IN THYROID TISSUES FROM RATS TREATED WITH A TUMORIGENIC AND A NON-TUMORIGENIC TRIAZOLE CONAZOLE FUNGICIDE.

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Conazoles are triazole- or imidazole-containing fungicides that are used in agriculture and medicine. Conazoles can induce thyroid follicular cell adenomas in rats after chronic bioassay. The goal of this study was to identify pathways and networks of genes associated with thyroid cancer. We compared transcriptional profiles from rats treated with a tumorigenic and a non tumorigenic conazole. Triadimefon, a rat thyroid tumorigen, and myclobutanil, which was not tumorigenic after a 2 year bioassay, were administered in the feed to male Wistar/Han rats for 30 or 90 days similar to the treatment conditions previously used in their chronic bioassays. Thyroid gene expression was determined using Affymetrix GeneChips (Rat 230_2). Gene Set Expression Analyses clearly separated the tumorigenic treatments (tumorigenic response group, TRG) from the non-tumorigenic treatments (non-tumorigenic response group). Core genes from these data sets were mapped to canonical, metabolic, and GeneGo processes and these processes compared across group and time. Extensive analyses were performed on the 30d genesets as they represented the major perturbations. Genesets in the 30d TRG group had over representation of fatty acid metabolism, oxidation, and degradation processes (including PPARγ and CYP involvement), and of cell proliferation responses. Core genes sets were combined into networks and found to possess signaling interactions. In addition, the core genes in each geneset were compared with genes known to be associated with human thyroid cancer. Among the genes that appeared in both rat and human data sets were: Acaca, Asns, Cebpg, Crem, Ddit3, Gja1, Grn, Jun, Junb, and Vegf. These genes were major contributors in the previously developed network from triadimefon-treated rat thyroids. It is postulated that triadimefon induces oxidative response genes and activates the nuclear receptor, Pparγ, initiating transcription of gene products and signaling to a series of genes involved in cell proliferation. This abstract does not necessarily reflect EPA policy.

1325 CHLORACETANILIDE HERBICIDE STRUCTURE-ACTIVITY STUDY USING BIOCHEMICAL AND GENOMIC APPROACHES.

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Alachlor and butachlor are chloracetanilide herbicides that are associated with olfactory carcinogenesis in rats following chronic exposure, whereas propachlor is not. We used biochemical and genomics approaches to understand what processes may be occurring in common between alachlor- and butachlor-treated rats, but not in propachlor-treated rats. Rats were treated with alachlor (126 mg/kg/day dietary equivalent) or with butachlor or propachlor at 3,000 ppm in the diet for periods up to 3 months. We measured glutathione levels in liver and olfactory mucosa (OM), MMP2 activation in the OM by zymography, and alterations in OM gene expression by microarray analysis. Glutathione levels were markedly decreased in alachlor- and butachlor treated rat OM following 10 days of treatment, but only alachlor treatment was significantly different from control. All three chloracetanilides caused MMP2 activation, contrary to our hypothesis that MMP2 activation is pivotal in the chloracetanilide carcinogenic response. There were a number of genes that were similarly regulated in alachlor- and butachlor-treated rats, but not so in propachlor-treated rats. These included alcohol dehydrogenase, aldehyde oxidase, glutathione S-transferase A3, olfactory specific medium-chain acetyl CoA synthetase, and interferon stimulated exonuclease gene 20-like 1 (predicted). The most significantly regulated gene in the study was vomeromodulin, which was dramatically upregulated by alachlor (>40-fold) and butachlor treatment, and less so in propachlor-treated rats. In summary, other than several instances of concordance of gene expression in alachlor and butachlor-treated rat OM, which differed in propachlor-treated rats, we did not identify a clear cut difference in response that would predict carcinogenic activity, but this may be due to the early time points evaluated in this study. Supported in part by NIEHS grant ES-08799 (MBG).

R 1326 HAZARD *VS.* RISK FOR CHEMICAL REGULATION.

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Different governmental organizations, and even different industries, have taken different approaches to decision-making about the safety of chemicals. U.S. and Canadian regulatory agencies take a risk-based approach to chemical regulation,

whereas the E.U. approach is hazard-based. Many retailers of products that contain chemicals, such as WalMart, are also starting to adopt a hazard-based expectations for their suppliers. Both approaches have their supporters, and both have positive and negative aspects. They may lead to very different decisions regarding acceptability based on the same data set. Since these decisions have important public health and economic impliations, it is important to understand more about them. The purpose of this Roundtable will be to present the current lay of the land in chemical regulation, and to highlight the differences in decisions that are often made by the two approaches, as well as their consequences.

R 1327 RECONCILING SCIENTIFIC AND ETHICAL CONCERNS IN THE USE OF ANIMALS FOR TOXICOLOGICAL RESEARCH.

T. A. Lewandowski. *Gradient Corporation, Seattle, WA.*

This roundtable will explore the tension between scientific necessity and ethical concerns regarding the use of animals in toxicological research. At present, scientific research depends on animal studies to address questions about efficacy and safety of potential drugs, and the potential hazards of chemicals. The Society of Toxicology and other societies have addressed ethical issues of using animals in research, and government regulations specifically address the scientific and ethical treatment of animals. In addition, there are ongoing efforts to develop alternative methods that could replace, refine, or reduce the use of experimental animals in toxicological research. For example, new methods with more specific and less variable endpoints may enable experimental designs that require fewer animals. Following a summary of recent trends in the use of animals in research, this roundtable will discuss the report recently released by the National Research Council regarding efforts to develop alternatives to animal testing, and how these alternatives can be implemented. We will then examine how advances in disciplines such as genomics might contribute to reductions in animal use. We will also consider refinement alternatives as well as approaches for recognizing and minimizing distress that animals may experience in toxicity studies. Finally, this roundtable will discuss limitations on the use of animal alternatives for toxicity testing in the regulatory arena – both for drug safety testing and for support of human health risk assessments.

R 1328 TOXICOLOGY TRAINING NEEDS: NEW FACES AND NEW TOOLS FOR THE 21ST CENTURY.

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There is growing concern within the discipline of toxicology with regard to the projected retirement of experienced toxicologists and the toxicology training pipeline from both a numbers and depth of training perspective. This problem is only becoming more acute due to budgetary constraints, regulatory and policy shifts and the advent of new chemical and biological challenges resulting from innovative and technology driven product development. Toxicologists are being asked to address safety issues for novel nanotechnology products, plant-made pharmaceuticals and industrials, combination therapeutics and concerns related to potential chemical and biological terror agents in a timely and cost effective manner. To address these issues, the next generation of toxicologists will need new tools and cutting edge training, and they must be produced in sufficient numbers to effectively replace the current cadre of toxicologists as they retire. A necessary first step in assuring the availability of qualified toxicologists is to begin a dialogue among the various potential employers on projected current and future needs in toxicology and what type of training experience would be most advantageous as the discipline moves into the 21st century. There is also a need to develop a clear picture of the number of new toxicologists that will be needed over the next 10 years. This session will focus on the number of toxicologists and the required skill sets needed as discussed by representatives from the industry, academic, research, consulting and government sectors and will be a positive step forward by addressing these needs at a meeting where most of the concerned toxicologists and many of those responsible for training and hiring toxicologists will be in attendance.

IS 1329 CROSS CULTURAL UNDERSTANDING OF ASIAN AND WESTERN VALUES IN THE WORKPLACE.

T. Kawabata[1] and D. Auyeung-Kim[2]. [1]*Pfizer, Groton, CT and* [2]*Charles River Laboratories, Sparks, NV.*

The competition in developing your career as a scientist can be daunting at times. It is clear that being productive through hard work alone is not the answer. In order to meet your career goals, scientists must also develop leadership and people skills. The mastering of these skills may be particularly challenging for those scientists reared with cultural values that differ from their western workplace culture. The differences in cultural values may be manifested in misunderstanding and barriers or "glass ceilings" in the development of one's career. Asian scientists and their co-workers and managers need to better understand how Asian and Western cultural values differ and how such cultural values are directly connected to behavior and practices in the workplace. Many Asians for example, are taught to respect authority, not to toot their own horn and value harmony of the team. It is also important to understand of how different behaviors are perceived by their non-Asian managers and co-workers in the Western workplace and the potential impact that may ensue when behaviors are misunderstood. For instance, not speaking up in team meetings may be perceived as being not interested or having nothing valuable to add to the discussion. These misperceptions may result in organizations underutilizing their Asian employees and decreased team effectiveness. In addition, Asian scientists may experience limited upward mobility and lower job satisfaction. This background information will be covered by Sally Huang-Nissen, a consultant and trainer with extensive experience in the area of cross-cultural effectiveness and diversity training. This will be followed by a roundtable discussion by a panel of SOT members from different backgrounds and with different career paths (academia, industry, government). The roundtable discussion will focus on their personal experiences and how these scientists addressed these cultural differences in their careers.

IS 1330 TOXICOLOGICAL AND PUBLIC HEALTH CHALLENGES IN AFRICA.

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Paucity of data and lack of information exchange present major challenges to identifying feasible risk management options for toxicological challenges in Africa. The associated consequences are typified by current global health initiatives that are unintentionally excluding toxicological and public health concerns pertinent to the African continent. For example, concerns about nanoproducts may not be as relevant to Africans as would exposure to cyanide from widespread consumption of cassava as a staple food source. Two-thirds of the 900 million sub-Saharan Africans live in rural areas and rely on agriculture and other natural resources for sustenance causing shrinkage in the environmental resource base of the region. The resultant decline in biodiversity imposes an enormous strain on food safety, which frequently leads to dependence on food sources tainted with toxicants. In addition, large volumes of industrial chemicals associated with growth in the petro-chemical, agro-chemical, textile, mining, and food preservation industries raise concerns on their short- and long-term effects. Oil pollution is an important and controversial subject of discussion in Africa. The public health impacts of new pharmaceuticals such as anti-retroviral drugs, new classes of anti-malarial drugs, and the growing problems of illicit drug dumping in Africa are largely unknown. Most countries lack the resources needed to conduct relevant human health risk assessment and for making environmental risk management decisions. This session provides a platform to bridge the gaps by highlighting some specific toxicological problems facing the continent, engaging scientists conducting global health researches, and bringing to the table individuals who have been part of risk management practices in the developed countries. It is hoped that feasible approaches that take into consideration Africa's geopolitical and cultural divides will be identified, discussed and prioritized to guide future interventions.

S 1331 DEVELOPMENTAL EFFECTS OF HEALTH AND DISEASE: PERSISTENT EFFECTS OF TOBACCO SMOKE EXPOSURE.

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Children are exposed to tobacco smoke. In a study of 5,400 U.S. school aged children, 85% had measurable cotinine levels in their blood. Whether it is prenatal exposure to mainstream smoke or postnatal exposures to sidestream smoke, childhood exposures are most often involuntary exposures and can result in adverse health effects. This is not surprising as tobacco smoke contains a complex mixture of compounds with toxic and/or bioactive effects. Yet the long term outcomes of childhood exposures are still not well understood. The purpose of this session is to bring together the latest research on the effect of tobacco smoke on development of the neural, immune and respiratory systems and to highlight potential areas of future research. There is recent concern about the possibility of persistent adverse health effects of tobacco smoke exposure that continue into adulthood after exposures during development in either the pre or postnatal period. Persistent effects have been described for the lung, the immune system and for both behavior and neurobiology.

S 1332 EFFECTS OF ENVIRONMENTAL TOBACCO SMOKE ON THE DEVELOPING IMMUNE SYSTEM OF INFANT MONKEYS.

K. E. Pinkerton, L. Wang and J. P. Joad. *Center for Health and the Environment, University of California, Davis, Davis, CA.*

Exposure to environmental tobacco smoke (ETS) is associated with an increased incidence of allergic and infectious diseases among children, thought to be partly due to the immaturity of the immune system. We investigated the effect of ETS exposure on postnatal immune development during the first year of life in the nonhuman primate. Fifteen neonatal rhesus monkeys were studied through 13 months postnatal age. They were randomized into three groups: 1) filtered air (FA); 2) continuous ETS exposure beginning at gestation day 50 (perinatal ETS) and 3) exposure to ETS beginning at 6 months of age (6 mo ETS). Complete blood counts, lymphocyte subsets and mRNA levels of 12 cytokines in peripheral blood mononuclear cells (PBMCs) were measured. Two weeks of ETS exposure in the 6 mo ETS group significantly increased neutrophil numbers and PBMCs IL-6 mRNA levels. Longitudinal data analysis demonstrated FA controls had an age-related increase in IFN-g, IL-2, and an age-related decrease in IL-10, IL-13 and IL-1. Compared to FA controls, ETS treatment groups displayed a progressive decrease in IFN-g, less of an increase in IL-2, and an increase for IL-10. We conclude short-term exposure to ETS can induce an acute systemic inflammatory response in the neonate, and long-term exposure to ETS beginning in utero or at 6 months postnatal age can significantly alter immune effectors. Discussion will include 1) exposure to ETS may compromise normal immune system development, 2) the role of critical windows of susceptibility to exposure effects during development and 3) potential implications for future onset of diseases.

S 1333 ETS AND GENDER EFFECTS ON ALLERGIC ASTHMA.

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The incidence of asthma in childhood is higher in males than in females; after puberty the gender bias switches to females. Exacerbation of asthma in human females has been noted during the progesterone dominant phase of the reproductive cycle. Previously using a balb/c mouse asthma model we have shown that environmental tobacco smoke (ETS) serves as an adjuvant for a T helper 2 /IgE response. Further studies have demonstrated a gender bias for development of allergic sensitization in female mice exposed to ETS prenatally and neonatally. Presently we report a synergistic effect of ETS and progesterone on development of the asthmatic phenotype. Female balb/c mice were ovarectomized and implanted with either progesterone pellets or placebo. Mice were exposed to either ETS of filtered air and were either sensitized with house dust mite allergen or placebo. Serum IgE levels, cytokine concentrations, bronchoalveolar lavage eosinophilia, lung histology, immunoperoxidase detection of IgE containing cells and progesterone receptor expression, and the extent of mucus metaplasia were examined. These parameters of asthma showed the greatest increase in mice exposed to both ETS and progesterone, while both ETS and progesterone also showed individual effects. We conclude that exposure of asthmatic individuals to ETS and concurrent treatment with exogenous progesterone may lead to enhanced allergic sensitization and exacerbation of asthma.

S 1334 PRENATAL EXPOSURE TO TOBACCO SMOKE INDUCES ASTHMA-RELATED RESPONSES IN NON-SENSITIZED FEMALE OFFSPRING LATER IN LIFE.

J. T. Zelikoff, S. Doherty, C. Hoffman and E. Brush. *School of Medicine, Department Environment Med., New York University, Tuxedo, NY.*

More than 70 million babies are born worldwide each year to mothers who smoked while pregnant. While past emphasis has been on immediately obvious perinatal consequences (e.g., preterm delivery and low birth weight), smoking during pregnancy has emerged as a risk factor for later onset disease outcomes in the prenatally exposed offspring. In addition to diabetes and cardiovascular disease, asthma, in the prenatally exposed offspring, has been repeatedly linked in epidemiological studies with maternal smoking. Most popular is the notion that early exposure to cigarette smoke acts to exacerbate, rather than induce, atopic outcomes such as airway hyperresponsiveness in the progeny. For this presentation, data will be presented that demonstrate that prenatal exposure of mice to a relevant concentration of mainstream cigarette smoke can, in and of itself, induce atopic outcomes including allergic antibody production and enhanced airway reactivity in juvenile and adult offspring not previously sensitized with allergen. Also discussed will be the persistence and gender-dependent nature of the effects.

S 1335 ENVIRONMENTAL TOBACCO SMOKE (ETS) AS A RISK FACTOR IN CHILDHOOD BEHAVIORAL DISORDERS.

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Cognitive ability, school performance, conduct disorders, and auditory processing are among the children's neuropsychological outcomes associated developmental ETS exposure in a number of human epidemiologic studies. Animal studies, which are valuable in supporting causality, identifying effective amounts of exposure and uncovering mechanism, are very limited. This presentation will discuss what we still need to know to effectively address ETS impact on this aspect of children's environmental health and will present new results that support the view that perinatal ETS exposure has adverse effects on cognitive function in a nonhuman primate model.

S 1336 MATERNAL SMOKING AND FETAL LUNG DEVELOPMENT.

E. R. Spindel. *Division of Neuroscience, Oregon National Primate Research Center, Oregon Health & Science University, Beaverton, OR.* Sponsor: L. Van Winkle.

Maternal smoking during pregnancy leads to permanently impaired lung function in offspring. Multiple studies have demonstrated decreases in pulmonary function in children whose mothers smoked during pregnancy, with the most sensitive indicator being decreases in forced expiratory flows. These decreases are evident in premature infants to high school students whose mothers smoked during pregnancy. Data from our laboratory indicate that nicotine crossing the placenta to interact with nicotinic receptors (nAChR) in developing lung is the key factor to alter lung development to lead to decreased pulmonary function. In developing lung, multiple nAChR subtypes are expressed including $\alpha3$, $\alpha4$, $\alpha5$, $\alpha7$, $\alpha9$, $\alpha10$, $\beta2$ and $\beta4$. In monkeys treated with nicotine throughout pregnancy, offspring showed identical decreases in forced expiratory flows as seen in humans whose mothers smoked during pregnancy. Offspring of nicotine-treated monkeys showed elevated levels of $\alpha7$ nAChR in airway epithelium and fibroblasts. Collagen levels were elevated in the airway fibroblasts in the identical distribution as the increases in $\alpha7$ nAChR expression. This suggests that nicotine upregulates $\alpha7$ nAChR expression in fibroblasts leading to increased collagen and altered lung function. This key role for $\alpha7$ nAChR in mediating the effects of nicotine was confirmed using $\alpha7$ knockout mice. In wildtype mice, combined prenatal and neonatal nicotine exposure decreased forced expiratory flows, but had no effect on pulmonary function in $\alpha7$ knockout mice. Because chronic nicotine can activate or inactivate nicotinic receptors depending on the tissue and receptor subtype, the effect of chronic nicotine on nicotinic currents in whole cell recordings of cultured bronchial epithelial cells was determined. This showed that nicotine activates and upregulates nAChR function in lung. Thus prenatal nicotine exposure acts to increase nicotinic signaling in developing lung which leads to altered lung development and alterations in offspring pulmonary function.

S 1337 SEX DIFFERENCES IN ADULT SUSCEPTIBILITY TO TOXIC CHALLENGE: ROLE OF NEONATAL ETS EXPOSURES.

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ETS alters the biology of conducting airways in children, increasing wheezing, cough and the incidence of SIDS. However, little is known about the effects of ETS exposures on the biology of the developing airway epithelium. To address the hypothesis that an early life ETS exposure alters the airway epithelium, we exposed male and female mice to ETS starting on gestational day 6 through adulthood. Distribution and abundance of CYP2F2, and glutathione S-transferase (GST) alpha, mu and pi enzymes were defined using immunohistochemistry and real time RT PCR. ETS elevated GSTs alpha and mu in both sexes. CYP2F2 was increased in female, but not male, mice exposed to ETS, particularly in terminal bronchiolar epithelium. GST pi expression was lower in the ETS exposed females than in males. To test whether this enzyme profile would result in elevated susceptibility, mice with a history of pre and postnatal ETS exposure were challenged with 200 mg/kg naphthalene i.p. Female mice exposed to ETS were more than 2 fold more susceptible than males to naphthalene injury. To further evaluate which other genes/proteins persist as altered until adulthood and may contribute to susceptibility to toxic challenge, we analyzed gene expression using oligonucleotide arrays on microdissected airway samples from: pre and postnatal FA (FA/FA), prenatal TS + postnatal FA (TS/FA), pre and postnatal TS (TS/TS) mice. In males, the TS/FA group contained more unique up regulated (727) and down regulated genes (501) than the TS/TS (295; 172) male group. 1134 genes were up or down regulated in common. In females, the TS/FA group contained more unique up regulated (86) and down

regulated genes (291) than the TS/TS (5; 30) female group. 126 genes were up or down regulated in common. We conclude that neonatal ETS exposure, including exposures that encompass only the prenatal period, alter airway epithelial specific gene expression and susceptibility to toxic challenge in a sex specific manner. Funding: ES013066, ES05707

S 1338 UNUSUAL MANIFESTATIONS OF ON-TARGET AND OFF-TARGET TOXICITY: TOXICITY OF KINASE INHIBITORS.

B. Car[2] and K. L. Kolaja[1]. [1]*Discovery and Investigative Safety, Roche, Palo Alto, CA and* [2]*Discovery Toxicology, BMS, Princeton, NJ.*

Abstract: In recent years, several relatively selective tyrosine and serine-threonine kinase inhibitors have been discovered and developed for therapeutic applications in oncology and immunology. In addition the kinome is being explored for targeted inhibition in a much wider variety of disease areas. Typically, small molecules or antibodies are used to target a kinase proximal or otherwise key to a signal transduction cascade leading to endpoints such as tissue inflammation or cell proliferation. In addition to toxicity mediated by biotransformation to toxic intermediates (e.g. hepatic and renal toxicity), a particularly broad and novel variety of adverse behavioral or tissue-based changes are noted with kinase inhibitors from memory loss (MEK), and extreme neuropathic pain (Ret), to failure of growth plate closure (VEGFR2), clastogenicity (many kinase inhibitors), cardiotoxicity, hematopoetic toxicity, and teratologic outcomes (Wnt kinases and many others). These effects may be mediated either by inhibition of targeted kinases, closely related kinases or ostensibly nonhomologous kinases to those targeted or a combination of on- and off-target effects. As more data are collected, an emerging trend is that the inhibition of kinases by parent molecules and their metabolites is the causative factor leading to the panoply of untoward toxicity. Substantial efforts requiring novel approaches in predictive toxicology, risk assessment and monitoring have been employed to adequately avoid, understand, and manage the outcomes of kinase inhibition. This Symposium summarizes the different experiences of several pharmaceutical companies in managing a challenging new area of the toxicology of pharmaceutical agents.

S 1339 INCREASED ORGAN CHEMICAL BURDEN AS A CONTRIBUTOR TO THE TOXICITY MECHANISM OF A RECEPTOR TYROSINE KINASE INHIBITOR IN RATS.

H. Hamadeh. *Toxicology, Amgen Inc., Thousand Oaks, CA.*

Kinase inhibitor toxicity was investigated the differences between two related receptor tyrosine kinase inhibitors with markedly different toxicity profiles. Interestingly, the two compounds have similar chemical structures, inhibit receptor tyrosine kinases in a similar fashion, and have similar pharmacological efficacious effects, yet reveal distinct toxicity profiles in Sprague Dawley rats. It was concluded that differences in pharmacokinetic properties of the toxic and non-toxic analog, such as volume of distribution, half-life, and organ concentrations, lead to a marked differences in chemical burden on target organs, and may have contributed to the vast differences in toxicity profiles with the two, otherwise similar molecules. The presentation will utilize this case study to highlight the challenges and learnings related to kinase mediated toxicity.

S 1340 KINASES OF TOXICOLOGICAL CONCERN: DEVELOPMENT OF *IN VITRO – IN VIVO* CORRELATIONS FOR KINASE INHIBITION AND TOXICITY.

K. L. Kolaja. *Discovery and Investigative Safety, Roche, Palo Alto, CA.*

Novel molecular and cell biologic approaches for detecting desirable and undesirable consequences of kinase inhibition will be explored with specific examples relating to bone marrow, cardiotoxicity, and clastogenicity. Through the use of kinase inhibition screening and retrospective analysis of in vivo toxicity data across the entire portfolio, several informative conclusions regarding kinases and their toxicological effects have been learned. The strategies and data discussed will provide practical approaches to early Discovery intervention/screening as it relates to in vitro methods of assessment of kinase inhibitor toxicology.

S 1341 DISCOVERY AND *IN VIVO* APPROACHES TO EVALUATION OF DIVERSE KINASE INHIBITOR TOXICITIES.

B. D. Car, B. Gemzik, A. K. Gupta, P. C. Levesque, D. Li and R. A. Westhouse. *Bristol-Myers Squibb Company, Princeton, NJ.*

In addition to the usual palette of small molecule-related toxicities, the safety assessment of kinase inhibitors yields occasional results of potential concern for patients that fall well outside the drug-related effects to which most toxicologists are accustomed. As N-rich, often cationic lipophilic heterocycles competitive with the ATP-binding site of kinases, ion channel interactions are expected and commonly observed with kinase inhibitors. In addition, interactions with adenosine receptors (A2A, A3A) appear to be over-represented with the potential consequence of altered coronary blood flow. Particularly important is the early evaluation of candidate molecules in stably transfected cells expressing cardiac K+, Na+. L-Ca++ channels, and study in rat or rabbit telemetry models for potential hemodynamic and electrocardiographic alterations. The signaling cascade leading to Erk phosphorylation in the hippocampus is interrupted by MekP42/44 kinase inhibitors resulting in abrogation of long term potentiation of hippocampal neurons, leading to a loss of short term memory acquisition. Behavioral studies employed to understand this potential effect will be described. Critical to the assignment of toxicologic effects of specific kinases is the evaluation of the phosphorylation state of downstream targets of suspect kinases in affected tissues. The importance of partnering kinase inhibitor risk assessment work with biologists and pharmacologists expert in their field is critical to optimal risk assessment in this new field of toxicology.

S 1342 EVALUATING MECHANISM- AND NON-MECHANISM-BASED TOXICITY OF KINASE INHIBITORS: P38 MAP KINASE INHIBITORS AS A CASE STUDY.

D. Morris. *Drug Safety R&D, Pfizer Global Research & Development, Chesterfield, MO.*

P38 kinase (p38K) is a member of the mitogen-activated protein kinase (MAPK) family and is a pivotal member of a signal transduction cascade leading to the regulation of cytokines in a variety of inflammatory cell types. Inhibitors of this enzyme are currently being evaluated as a potential disease modifying treatment for rheumatoid arthritis and other chronic inflammatory diseases. In preclinical toxicology testing of a series of p38 kinase (p38K) inhibitors in the rat, a number of general and organ toxicities were identified, including CNS, renal, gastrointestinal tract, liver, pancreas and adrenal toxicity. Organ toxicities were characterized histologically as including cellular degeneration and frank necrosis, with and without focal to multi-focal inflammation. To investigate the mechanism and role of p38 kinase in these observed organ toxicities, kinase selectivity screening and an in vitro model using primary rat hepatocytes were established to further characterize the toxic effects of the compounds. Results of these studies demonstrated that organ toxicity with the various compounds, when corrected for free fraction plasma exposures, were not correlated with p38 kinase inhibition or any other kinase evaluated in selectivity screens. In contrast, toxic effects (incidence and severity) in vivo were found to have a strong correlation with in vitro cytotoxicity in rat hepatocytes, with a correlation coefficient of 96%. Of note was the observation that toxicity in rat hepatocytes did not predict for the occurrence of liver toxicity in vivo. This in vitro model was subsequently used to identify the structure-toxicity-relationships of the chemistries associated with organ toxicity and to prioritize lead candidates devoid of overt toxicity in subchronic rat toxicology studies. A description of the experimental approaches used in evaluating the safety of p38K inhibitors, and implications for kinase inhibitor safety assessment in general, will be discussed.

S 1343 SPECIES-SPECIFIC TOXICITIES OBSERVED WITH KINASE INHIBITORS: IMPACT ON THE PRECLINICAL SAFETY PLAN AND REGULATORY STRATEGY.

J. W. Davis. *DSRD, PGRD, Chesterfield, MO.*

Anti-TNF alpha therapies have been a focus for the treatment of rheumatoid arthritis (RA), most of these being biologics. The p38 kinase (p38k) pathway is chiefly responsible for the inflammatory reaction that results in the induction of TNF alpha. Small molecule inhibitors have been pursued as an alternative to more expensive biologics. A number of toxicities have been reported clinically and pre-clinically that have limited their advancement into late-stage clinical studies. Hepatic and skin toxicities are chief among these target organ toxicities, which are likely to be a direct consequence of p38k inhibition. Furthermore, exploratory acute toxicity studies of p38k inhibitors in beagle dogs demonstrated single dose toxicity (apoptosis/necrosis) to mucosal and peripheral lymphoid tissues. Similar lymphoid toxicity was not observed in Sprague-Dawley rats or cynomolgus macaques at exaggerated exposures

in exploratory toxicology studies. Immunohistocytochemical studies have demonstrated that the dog was uniquely sensitive to this effect due to the overexpression of p38 in resident B lymphocytes, a pattern that is not observed in other species. Additionally, in vitro B cell proliferation assays indicate that exposure of canine B cells to p38k inhibitors results in cytotoxicity while rodent, non-human primate and human B cells are not sensitive to this effect. Downstream targets in the p38k pathway directly responsible for TNF alpha message stabilization have also been evaluated and similar effects have been observed further implicating p38k inhibition as the primary mechanism for lymphoid toxicity in dogs. The results of these investigations led to the selection of cynomolgus macaques as the second species for safety evaluation. Furthermore, the toxicity was determined to be monitorable, reversible and irrelevant to humans which allowed for advancement into the clinic.

W 1344 NATURAL KILLER CELLS AS TARGETS OF DRUGS, TOXICANTS, AND BIOLOGICALS.

R. Krishnaraj. *Pharmacology, University of Illinois at Chicago, Chicago, IL.*

NK (natural killer) and NK-T cells are immunologically important cells involved at the interphase of innate and adaptive immunity and respond to physiopathological processes. They may even be more sensitive than some of the other commonly used immunotoxicity tests. Suppression of NK cell activity by environmental contaminants is well known e.g., organotins. NK cell activity is one of the initial screening study end points for immunotoxicity testing under European drug regulatory guidelines. The pharmacological activation of NK cells in anti-viral and anti-tumor therapies has been emerging for approved therapeutics and those under clinical trial. Imiquimod, a TLR7/8 ligand that induces NK-activating cytokines is an approved agent for genital warts caused by papilloma virus. NK cell modulations are known during Herpes virus and Cytomegalovirus infections. Studies showing NK or NK-T cells being recruited into the liver after viral infection, NK cells moving into the lymph nodes in response to immunization or tumors, participation of NK / NK-T cells in inflammatory responses in liver and lungs are noteworthy. The therapeutic activity of monoclonal antibodies against human cancer cells may involve antibody-mediated cell-mediated cytotoxicity, one of NK cells' known functions. Several natural product extracts modulate NK cells. Thus, the effects of immunotoxicants, immunotherapeutics or small molecules on NK cells, the modulations of NK cells in anti-infectives / vaccine development are of interest to toxicologists and drug discovery groups. A brief overview of the functions, and concepts of NK cells will be followed by some of the cutting edge results from in vitro and in vivo data from different species.

W 1345 ENVIRONMENTAL IMMUNOTOXICANTS ON HUMAN NK CELLS.

M. Whalen. *Chemistry, Tennessee State University, Nashville, TN.* Sponsor: R. Krishnaraj.

NK cells are responsible for limiting the spread of blood-borne metastases as well as limiting the development of primary tumors. Thus, any agent that interferes with the anti-tumor ability of NK cells could increase the risk of tumor incidence and/or viral infections. Assessment of human NK function in response to both environmental contaminants and pharmaceuticals may be an important indicator of the long term harm presented by these compounds. When a compound is found to interfere with the immune function of the NK cell it is further examined for its capacity alter the biochemical pathways needed by the NK cell to carry out its functions. This involves monitoring the effects of the compound on the expression of particular proteins and the enzymatic activity of key signaling proteins. Some of the compounds that we have investigated (Organotins, Tributyltin and Dibutyltin) in vitro are in the same range as seen in vivo.

W 1346 NATURAL KILLER CELLS AT THE INTERPHASE OF INNATE AND ADAPTIVE IMMUNITY.

C. Munz. *Laboratory of Viral Immunobiology, The Rockefeller University, New York, NY.* Sponsor: R. Krishnaraj.

Cells of the innate immune system act in synergy to provide a first line of defense against pathogens. Dendritic cells (DCs) stimulate proliferation, cytokine secretion and cytotoxicity of Natural Killer (NK) cells prior to the activation of adaptive immunity. We could demonstrate that DCs matured with double-stranded RNA produced high levels of NK cell-stimulating IL-12 and thereby activated NK cell proliferation and cytokine secretion more efficiently than any other mature DC preparation. Primarily immunoregulatory CD56brightCD16- NK cells responded to DC activation, and the same NK cell subset is enriched in human secondary lymphoid tissues, including tonsils, the primary site of infection with the Epstein-Barr virus (EBV). In line with a direct anti-viral function of NK cells in secondary lymphoid organs, DCs elicited 50-fold stronger interferon-gamma secretion by tonsillar NK cells than by peripheral blood NK cells, reaching levels that inhibited EBV-induced B cell lymphomagenesis. In fact, 100-fold less tonsillar than peripheral blood NK cells were required to achieve the same control over EBV-transformed B cells in vitro. These results suggest that NK cell activation by DCs limits primary EBV infection in tonsils until adaptive immunity establishes immune control of this persistent and oncogenic human pathogen.
These studies are relevant for dendritic cell vaccine efficiency with respect to innate immune response activation and polarization of adaptive immune responses via activated Natural Killer cells.

W 1347 DIRECT AND INDIRECT EFFECTS OF ETHANOL ON ACTIVATION OF NK CELLS THROUGH TLR3.

S. B. Pruett. *Department Basic Sciences, Coll. Vet. Med., Mississippi State U., Mississippi State, MS.*

Poly inosinic-polycytidylic acid (Poly I:C) is a synthetic analogue of viral double stranded RNA. It activates protective innate resistance to viruses and cancer cells in vivo. Ethanol (EtOH) decreases host resistance to viral infections and cancer, and the purpose of the present study was to extend previous studies designed to determine the mechanisms by which acute EtOH administration alters poly I:C-induced activation of innate immunity. EtOH decreases TLR3-mediated cellular signaling, including the activation of NF-kappaB. However, those studies were done after removal of cells from mice. In experiments reported here, NF-kappaB luciferase transgenic mice were used to determine if this inhibition also occurred in vivo. Mice were treated with EtOH (4-6 g/kg by oral gavage) and then treated 30 min later with poly I:C. Thirty min to 4 hr later, luciferase activity was evaluated by injecting luciferin and imaging the mice using the IVIS system, which quantifies luminescence. EtOH profoundly inhibited NF-kappaB driven luciferase expression 30 min after poly I:C, and significant inhibition was observed at 2 hr but not at 4 hr. These results indicate that profound suppression of NF-kappaB activation could be an important mechanism by which EtOH alters TLR3 mediated innate immunity, and this was associated with decreased resistance to B16F10 tumor cells. The role of glucocorticoids in feedback inhibition of cytokine production was examined and changes in cytokine production were substantial but not entirely consistent with glucocorticoid-mediated effects (as indicated by inhibition of corticosterone synthesis or activity). Finally, microarray analysis of mice treated with poly I:C or poly I:C + EtOH was compared to results for mice treated with live Escherichia coli with or without EtOH. The results suggest relatively few common effects of EtOH with both stimuli, which was surprising since TRIF signaling is utilized by both TLR3 and TLR4. This opens the possibility that signaling effects of EtOH are different for different TLRs. This work was supported by NIAAA grant AA0019505.

W 1348 SUBTYPE MARKERS DEFINE THE TISSUE SPECIFIC EFFECTS OF CHRONIC ALCOHOL CONSUMPTION ON NK CELLS.

G. G. Meadows and H. Zhang. *Cancer Prevention & Research Center, Washington State University, Pullman, WA.*

Chronic alcohol consumption reduces peripheral natural killer (NK) cell numbers and compromises NK cell cytolytic activity in the spleen; however, the underlying mechanism is not completely understood. It was recently found that the peripheral NK cell pool consists largely of bone marrow (BM)-derived and thymus-derived cells. These cells are phenotypically and functionally different. The effects of alcohol consumption on these subpopulations have not been studied previously. Using a well-established alcohol feeding model, we found that chronic alcohol consumption decreases the percentage and number of splenic NK cells, especially those expressing a mature phenotype. Alcohol consumption did not alter NK cells in the thymus. NK cells in the BM were significantly increased; however proliferation rate was not altered by alcohol consumption. Alcohol consumption increased CD127+ and decreased Ly49D+ NK cells in the spleen but not in the BM. Chronic alcohol consumption increased IFN-gamma producing NK cells and GATA-3 expression in splenic NK cells. The results indicate that chronic alcohol consumption perturbs the balance between thymus-derived and BM-derived NK cells in the spleen. The increased proportion of thymus-derived NK cells in the spleen likely results from impaired release of these from the BM.

W 1349 SAFE APPROACHES TO TOPICAL PRODUCT DEVELOPMENT.

W. G. Reifenrath. *Stratacor, Inc., Richmond, CA.*

Topical products are applied to the skin, eyes, or mucosal membranes for purposes that vary from altering appearance, to combating disease or to protecting against environmental assaults. Topical formulations are usually complex mixtures that may contain one or more pharmacologically active agents, a vehicle to facilitate ap-

plication, components to improve user acceptability, agents to promote or retard penetration or evaporation, stabilizers or preservatives, as well as other materials depending on the ultimate use. Most topical drug products are formulated with commonly used excipients that are included in FDA's Inactive Ingredients Guide. But some new products may need novel ingredients that offer benefits not available with commonly used excipients. The potential of a formulation to produce untoward effects is governed by the properties of each ingredient and the presentation of those ingredients to a site of action. However, simple addition of individual ingredient toxicities has proven inadequate for predicting the toxic potential of the formulation. Good toxicological judgment and critical testing thus play an important role in eliminating formulation problems well before they become significant clinical issues. Safety considerations are paramount for products intended for damaged tissue, and products for wound care or healing or for aging populations have unique requirements. Inclusion of in vitro studies to include skin penetration and distribution assessment, corrosion assays, and skin culture tests can aid in early stage toxicological decisions. The primary test systems for predicting skin corrosion or irritation have been the human cornified tissue constructs. The skin corrosion and basic irritation endpoints have recently completed formal validation. Assessment of eye irritation potential uses a range of test systems, with some systems tailored to demonstrate the required mildness of formulation. In summary, the judicious choice of excipients, the proper design of formulation, and the information gained from critical pre-clinical tests combine to make the difference between success and failure for a promising topical product.

W 1350 THE ROLE OF TOXICOLOGY IN TOPICAL DRUG DEVELOPMENT.

L. Mutter. *Preclinical, Regulatory Affairs, Dow Pharmaceutical Sciences, Inc., Petaluma, CA.*

Topical drug development requires toxicological evaluation of systemic as well as local toxicity. The systemic toxicology program does not need to be repeated for new formulations (e.g. 505(b)(2) NDA) of an approved drug. However, local tolerance and repeat dose topical (e.g. dermal, ocular, vaginal, etc) toxicity studies with the to-be-marketed formulation are required for each new drug product. Topical formulations are usually complex mixtures of excipients designed to deliver drug to the target while providing patient acceptance (cosmetic elegance) and product stability. Local toxicity, especially irritation, is of paramount importance for the successful development of a topical drug product that may be applied to injured tissue. Local tolerance testing, including in vitro alternative methods, may be used in early formulation development to guide selection of non-irritating excipients and formulation. In vitro models for irritation are available for most topical target including skin, ocular and vaginal tissue. Current regulatory guidelines for drug development are based on animal studies of local tolerance. Examples of nonclinical development programs for dermatological pharmaceuticals using both new chemical entities and product line extension examples will demonstrate the regulatory needs for topical drug development. Toxicology support in the early phase of topical drug development aids selection of a formulation that will be well tolerated in nonclinical and clinical studies for marketing approval.

W 1351 TOXICOLOGICAL CONSIDERATIONS FOR THE USE OF EXCIPIENTS IN TOPICAL DRUG PRODUCTS AND FDA'S EXCIPIENT GUIDANCE.

R. E. Osterberg. *Aclairo PDG Inc., Chevy Chase, MD.*

Topical drug product formulations usually contain a variety of excipients in addition to the active pharmaceutical ingredient(s) (API). These ingredients are necessary for a variety of purposes as some are essential to manufacture the drug product and others are necessary to make the product esthetically acceptable to the patient. Most topical drug products are formulated with commonly used and regulated excipients that are, for the most part, included in FDA's Inactive Ingredients Guide. However, some new products may need novel, new excipients that offer formulation benefits not available with the commonly used excipients. Novel excipients that have not been used in other topical drug or consumer products may have unknown and unacceptable toxicities. This presentation will discuss excipient toxicities and the types of toxicity information that the FDA describes in its Excipient Guidance that would be needed for a novel excipient's introduction into a topical product.

W 1352 *IN VITRO* APPROACHES FOR PREDICTING IRRITATION POTENTIAL OF TOPICAL EXPOSURES TO THE SKIN AND EYES.

J. W. Harbell. *Mary Kay Inc., Addison, TX.*

An essential part of topical product development is the determination of tissue irritation potential from intended and reasonably foreseeable use. For most products, the primary tissues of concern are the skin and eyes. The irritation potential of a formulation is governed by the toxicity of each ingredient and the presentation of those ingredients to the target tissue of interest. Simple addition of the individual ingredient toxicities has proven inadequate for predicting topical irritation. In vitro methods have been developed that can model the more complex interaction between the formulation and the target tissue. In some cases, these methods are used for formulation development/preclinical qualification. In other cases, they may be used as the definitive test when clinical confirmation is not appropriate. The primary test systems for predicting skin corrosion or irritation have been the human cornified tissue constructs. With a functioning stratum corneum, they can account for penetration of the formula components as well as their direct toxicity to the viable keratinocytes. The skin corrosion and basic irritation endpoints have recently completed formal validation. Assessment of eye irritation potential uses a range of test systems. The studies of Maurer and colleagues have shown the relationship between the initial depth of injury to the cornea and overall irritation potential. Thus, the in vitro test system must be able to model the depth of injury associated with the irritation. For most formulas, there is some expectation of the irritation potential. Some systems are tailored to demonstrate the required mildness of the formulation. These approaches include clonogenic assays (ophthalmic solutions) and cytotoxicity assays using extended exposures to the surface of human epithelial constructs (eye area cosmetics). Expected mild to moderate irritants may be resolved using short exposures to the human epithelial constructs while the moderate to severe irritants will require a full thickness cornea. In summary, these methods can provide useful predictions of irritancy potential of topical formulations.

W 1353 SAFETY AND MECHANISTIC CONSIDERATIONS IN SKIN TRANSPORT OF TOPICAL PRODUCTS.

W. G. Reifenrath. *Stratacor, Inc., Richmond, CA.*

Safety assessments of dermatological products are facilitated by an understanding of the disposition and fate of chemical entities after their application to the skin surface. Processes such as evaporation, permeation into different anatomic layers, and metabolism are inter-related and excessive penetration or distribution beyond the site of action can lead to untoward effects. Topical products are almost always mixtures, many times with complex vehicles, and their development typically involves large numbers of formulation candidates, too many to be screened for permeation in vivo or in individual diffusion cells. Instead, standard 24 well plates have served the purpose of multi-well diffusion assemblies to study transport into and through the skin, allowing over 50 diffusion experiments or cells to be run simultaneously. Electronic autoradiography of tape strips provides an efficient assay of drug diffusion into the stratum corneum. This method is particularly suited for actives whose site of action is the skin itself, such as anti-inflammatory, anti-fungal, anti-sense agents etc. Measures of skin metabolites in the receptor fluid can signal toxic effects. When more detailed study can focus on fewer formulations, or when the product is a transdermal, individual flow-through diffusion cells are used. Specialized evaporation-penetration cells allow the mass balance determination of volatile entities such as insect repellents, solvents or fragrances. Kinetic analysis of profiles of evaporation over time can be related to the performance of products, when efficacy is dependent on vapor phase concentrations. In addition, a controlled air flow over the skin allows the study of the effect of hydration of both product and skin on permeation. In summary, permeation and skin disposition data have proven useful for both safety and efficacy considerations in the overall process of topical skin product development.

W 1354 TOXICOLOGICAL CONSIDERATIONS IN THE FORMULATION AND DEVELOPMENT OF WOUND HEALING AND TISSUE REPAIR PRODUCTS.

D. W. Hobson. *H&H Scientific Services LLP, Boerne, TX.*

The development of products for tissue repair, burns and wound healing applications presents unique toxicological challenges that are quite distinct and different from the formulation and approaches to the evaluation of many dermatological products. Safety for these products should be carefully built in beginning with product design and formulation considerations specific to the intended conditions of treatment as well as any special patient and caregiver needs. Formulation approaches and components considered generally safe for many dermatologicals and cosmetics may not be applicable for products intended to be applied to wounded, burned or otherwise compromised or damaged skin. Aging of the tissues as well as infection and the presence of other pharmaceuticals in or near the treatment site may also be factors for consideration. This presentation will describe toxicological approaches toward development of topical products for chronic wounds such as diabetic ulcers, venous stasis ulcers and burns as well as practical methodologies for the safety assessment of wound healing and tissue repair products intended for use on aging populations or acute wounds from traumatic injury or burns.

W 1355 THRESHOLD OF TOXICOLOGICAL CONCERN: HISTORY, CURRENT USES AND FUTURE OPPORTUNITIES.

S. P. Felter and K. L. Blackburn. *Central Product Safety, Procter & Gamble, Cincinnati, OH.*

The Threshold of Toxicological Concern (TTC) approach was developed by the USFDA in the 1980s as a means to more efficiently address questions related to the safety of food packaging materials that have some potential to migrate to foodstuffs, but at levels that would result in exceedingly small human exposures. The approach is based on the fundamental premise that there is an exposure to untested chemicals below which adverse health effects will be negligible or absent. TTC-based exposure limits are established based on the distribution of potencies of chemicals for which toxicity data exist, with a tiered approach being developed that includes both cancer and non-cancer endpoints. For an untested chemical, a TTC-based exposure limit is then determined by placing the chemical into the appropriate tier based on evaluation of chemical structure, the presence of structural alerts, and other available data. This session will provide an overview of the history of TTC; the scientific basis behind the tiered TTC exposure limits; current regulatory acceptance of TTC; and future opportunities for the continued expansion of this risk assessment tool.

W 1356 HISTORY AND OVERVIEW OF THE THRESHOLD OF TOXICOLOGICAL CONCERN (TTC) CONCEPT.

A. McDougal. *CFSAN, U.S. FDA, College Park, MD.* Sponsor: K. Blackburn.

In July 1995, the U.S. Food and Drug Administration published a Final rule in the Federal Register establishing a ``threshold of regulation" process for determining when the likelihood or extent of migration to food of a substance used in a food-contact article is so trivial as not to require regulation of the substance as a food additive. The approach was based on the premise that at a sufficiently low level, exposure to components of food-contact materials could be assumed to pose a negligible risk to human health. That threshold was set at a dietary concentration of 0.5 parts per billion (ppb; equivalent to an intake of 1.5 ug/day) based on an evaluation of effects observed for hundreds of chemicals tested in both chronic and short-term animal feeding studies. Chemicals for which there is reason to suspect, based on test data or chemical structure, that the substance may be a carcinogen are excluded from this approach. This presentation will cover the history of the TTC concept and the scientific basis supporting FDA's "Threshold of Regulation."

W 1357 APPLICATION OF THRESHOLD OF TOXICOLOGICAL CONCERN (TTC) TO THE SAFETY EVALUATION OF FLAVORING SUBSTANCES.

T. Adams. *The Roberts Group, Washington, DC.* Sponsor: S. Felter.

For more than a decade the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has evaluated the safety of flavouring substances using a science-based procedure that incorporates the concept of Threshold of Toxicological Concern (TTC). Based on an extensive toxicity database (Munro et al., 1996) conservative TTCs have been derived for three Decision Tree (DT) structural classes (I, II, and III) of increasing toxic potential (Cramer et al., 1978). These conservative TTC limits have been used to screen chemical groups of structurally related flavouring substances to identify those having intake sufficient to warrant additional evaluation. More than 1700 substances have been evaluated to date. Based on the application of the TTC and a comprehensive review of the available safety data, no significant safety concerns exist for the use of flavouring substances under current conditions of intake.

In retrospect, the subset of toxicity data available for the different chemical groups of flavouring substances evaluated by the JECFA indicate that the different structural class TTCs are extremely conservative. A comparison of metabolism and toxicity data collected during the JECFA program can be used as a basis for revisions to the DT structural classes and further refinements in the toxicity database, both critical factors affecting threshold values. The impact of the revised structural classes and TTCs on the outcome of the evaluations is discussed.

W 1358 USE OF THRESHOLD OF TOXICOLOGICAL CONCERN (TTC) FOR GENOTOXIC IMPURITIES IN FOOD.

I. C. Munro and B. Danielewska-Nikiel. *CANTOX Health Sciences International, Mississauga, ON, Canada.*

The TTC is used by JECFA and EFSA for the safety evaluation of flavors. A question that arises in the application of the TTC to flavors, which also has ramifications for the general acceptance of the TTC for food safety evaluations, is whether the TTC can be applied to substances that are genotoxic or have structural alerts for genotoxicity. This issue also arises with pharmaceuticals which may contain low levels of genotoxic impurities. Müller et al. (Reg Tox Pharm 44:198, 2006) proposed a scheme to establish safe exposure levels for genotoxic impurities in drug products. For substances that are threshold-based genotoxins, they recommended exposure limits based on NOAELs, while for those lacking evidence for a threshold a TTC of 1.5 μg/day could be applied. Ball et al. (Toxicol Sci 97:226, 2007) proposed a lower safety concern threshold value of 0.15 μg/day for potentially genotoxic leachables in inhaled drug products. This threshold value is consistent with that suggested by Kroes et al. (Fd Chem Tox 42:65, 2004) for chemicals with structural alerts for potential genotoxicity present at low levels in the diet. Another consideration for the use of the TTC in safety evaluations is that genotoxicity observed in vitro may not be expressed in vivo. Noting this, JECFA uses TTCs for non-carcinogenic endpoints in the safety evaluation of flavors that may express genotoxicity in vitro, but lack genotoxicity in vivo. Clearly what constitutes definitive evidence for genotoxicity is key in the determination of when and when not to apply the TTC concept to genotoxic substances.

Looking to the future, there is a need to harmonize the divergent TTC approaches proposed for the safety evaluation of impurities in pharmaceuticals and food substances that possess genotoxic properties or have structural alerts for genotoxicty. There also is a need to develop criteria for the interpretation of genotoxicity data especially when exposures are extremely low and where evidence of genotoxicity in vivo is not convincing.

W 1359 USE OF THRESHOLD OF TOXICOLOGICAL CONCERN (TTC) APPROACH IN ASSESSMENT OF GENOTOXIC IMPURITIES IN PHARMACEUTICALS.

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The synthesis of pharmaceutical products often involves the use of reactive reagents as well as the formation of potentially reactive intermediates and by-products. Some of these reagents, intermediates or by-products may be present in the final drug substance and drug product as low level impurities. These impurities may be chemically reactive and have the potential for unwanted toxicities including genotoxicity and carcinogenicity. In this presentation, the concept of a staged threshold of toxicological concern (TTC) approach for assessing the virtually safe limits for the intake of genotoxic impurities over various periods of exposure will be described. In addition, the use of a procedure for testing, classification, qualification, toxicological risk assessment, and control of impurities possessing genotoxic potential in pharmaceutical products will be outlined

W 1360 THE THRESHOLD OF TOXICOLOGICAL CONCERN (TTC) – FUTURE OPPORTUNITIES.

A. G. Renwick. *School of Medicine, University of Southampton, Southampton, United Kingdom.* Sponsor: S. Felter.

The threshold of toxicological concern (TTC) is being used increasingly for the risk assessment of chemicals present in food at very low levels. The approach avoids the need for chemical-specific in vivo animal toxicity data by comparing the estimated intake of a compound with hazard characterization data for structurally-related compounds. The paper by Kroes et al. (2004) incorporates a decision tree in which the intake is compared with a series of escalating TTC values. Recently, the TTC approach has been applied to impurities in pharmaceutical products, household products and cosmetics. Application of the TTC approach to cosmetic ingredients and impurities requires relevant TTC values and a simple and pragmatic method of exposure assessment. Due to the absence of an extensive and systematic dermal toxicity database, specific TTC values cannot be determined for the topical route. A detailed analysis of route-dependent differences in first-pass metabolism showed that the oral TTC values, as used in the paper of Kroes et al. (2004), are valid for topical exposures. The physicochemical characteristics of the compound can be used to predict the relationship between the amount applied and the internal dose, which is compared with the relevant TTC value. The relationship between the pattern of cosmetic use and the internal dose can be taken into account by conservative default adjustment factors. The TTC approach relates to systemic effects, and would not provide an assessment of any local effects at the site of application. Application of the TTC approach to inhalation would require considerations of possible local effects and peak effects, and the development of a simple method of exposure assessment. Overall the TTC approach provides a useful, simple and flexible method for the safety evaluation of compounds of known chemical structure in the absence of chemical-specific toxicology data.

Kroes, R., et al. 2004. Food and Chemical Toxicology, 42, 65-83.

W 1361 USE OF BEHAVIORAL AND NON-ROUTINE NEUROLOGICAL APPROACHES IN DRUG DISCOVERY TOXICOLOGY.

B. Gemzik[1] and K. Steinmetz[2]. [1]*Discovery Toxicology, Bristol-Myers Squibb Co., Princeton, NJ and* [2]*SRI international, Menlo Park, CA.*

Novel pharmaceutical targets, including kinase inhibitors for inflammatory diseases and oncology, have been evaluated for their potential as therapeutic interventions in recent years. Unusual considerations for the toxicologist may include unwanted neurobehavioral effects, and deficits in learning and memory acquisition. In addition, interactions of chemical structure types with biogenic amine (e.g., serotonergic and dopaminergic) receptors and their metabolism and uptake mechanisms are relatively common off-target effects. Toxicologists in the drug discovery environment are confronted with the adverse neurobiology of small molecules and are required to place these effects in a broad risk-assessment perspective. Bridging pharmacology and toxicology, this expertise is of growing interest in the toxicology community - this session focuses the current knowledge and awareness in an area traditionally considered peripheral to the issues that most toxicologists are asked to address. The goal of this session is to raise awareness to the role of toxicologists in defining adverse neurological effects, particularly within the discovery and target selection environment, through illustration of this field, the techniques employed, and with specific examples of risk assessment for neurobehavioral and related toxicity.

W 1362 NOVEL CNS PHARMACOLOGIC TARGETS: CONSIDERATIONS FOR EVALUATING ADVERSE BIOLOGY.

G. J. Carey. *Neurobiology, Microbia Inc., Cambridge, MA.* Sponsor: B. Gemzik.

The primary focus of the discovery of novel CNS pharmaceutical targets has been on target validation and characterization. The potential for adverse biology (sensory/excitatory, affective, cognitive, abuse/addiction, neuroendocrine regulation) associated with the target or as a consequence of relative non-selectivity, both close and further removed from the target, represents a largely novel consideration in early discovery outside of CNS research. This presentation will provide awareness and describe challenges, with perspective and examples from the point of view a discovery pharmacologist.

W 1363 LEVERAGING NEUROBEHAVIORAL ASSESSMENT OF ADVERSE CNS EFFECTS TO IMPROVE CANDIDATE SELECTION.

M. J. Kallman. *Investigative Toxicology, Lilly Research Labs, Greenfield, IN.*

A systematic observational/neurobehavioral characterization provides a broad functional assessment that can be particularly useful in the initial evaluation of a suspected liability. This presentation will detail a modified Irwin-type assessment in rodents and its use in evaluation of novel compounds of interest at the early discovery biology/toxicology interface. Examples of the application of the Irwin evaluation and other more quantitative assessements of motor function will be provided to illustrate strategies for improving candidate selection.

W 1364 THE EFFECTS OF KINASE INHIBITORS ON LONG-TERM POTENTIATION AND MEMORY.

C. A. Miller and D. Sweatt. *Neurobiology, University of Alabama, Birmingham, AL.* Sponsor: B. Gemzik.

In the process of mapping the signaling pathways that underly the neuronal development and maintenance of memories, the learning and memory field has discovered important roles for a number of kinases, as well as enzymes previously appreciated only for their involvement in the regulation of epigenetic mechanisms during development and cancer tumorigenesis. This presentation will focus on the requirement of ERK/MAP kinase, histone deacetylase and DNA methyltransferase activity for hippocampal neuronal plasticity. Small molecule inhibitors of all three of these proteins interfere with normal memory formation. Systemic exposure or exposure through site-specific infusion of the compound directly into the hippocampus can affect target activation profiles in the hippocampus and this will be discussed in terms effects on associative learning assays and on an in vitro analog of memory formation, long-term potentiation.

W 1365 MAXIMIZING SENSITIVITY AND EFFICIENCY IN THE DETECTION OF PROCONVULSANT LIABILITY IN RODENTS.

M. Bell. *Safety Assessment, AstraZeneca Pharmaceuticals, Wilmington, PA.* Sponsor: P. Ciaccio.

Seizure represents a significant liability of pharmaceuticals. A review of the 2003 PDR indicates that more than 100 drugs demonstrate a low incidence (<3%) of seizure in man; a smaller number of compounds are associated with a high incidence (>3%) of seizure. Typically, the evaluation of a compound for seizure potential occurs late in the discovery process with convulsion in an animal as the first indication of risk. However, strategic use of available data and different bioassays can be used throughout preclinical discovery to increase sensitivity and efficiency of seizure detection. Clues to proconvulsant liability for a compound may be revealed by existing literature on the target (of compound chemistry), secondary pharmacology, ADME properties, and behavioral observations from initial in vivo studies. In silico strategies against convulsant liability have met with some success, and may improve as interactions with specific ion channels and receptors are better understood. An understanding of in vitro receptor and ion channel binding and functional effects is useful in assessing proconvulsant risk. Because seizure per se is a system event, models that include system properties are more likely to accurately predict clinical risk. In vitro brain slice electrophysiology models complement EEG models in understanding mechanism of action, especially in the hippocampus. In vivo methods are limited by the balance between monitoring methods and the subject's response to invasive measurement. Seizure detection based on severity of motor symptoms alone overlooks nonconvulsant seizures. EEG is superior in sensitivity, but implantation of probes can increase the body's inflammatory response and lower seizure threshold. An assessment must ultimately be interpreted in the context of the therapeutic indication. Specific models based on behavioral observation and EEG (with or without a standardized precipitant) and strategic use of different proconvulsant bioassays to increase sensitivity and efficiency of detection in project case histories will be discussed.

W 1366 THE PHENOTYPIC EVALUATION OF NEUROBEHAVIORAL ACTIVITY IN KNOCKOUT MOUSE MODELS.

D. Brunner. *Behavioral Research and Development, PsychoGenics Inc., Tarrytown, NY.* Sponsor: B. Gemzik.

The development of new therapeutics, especially acting through novel proteins is critical to the improved treatment of many disorders. However, this novelty is often attended by a lack of experience with the effects of modulating these targets in many tissues. Preclinical evaluation within particular therapeutic area groups generally focuses on the effects in a particular organ system. As drug discovery becomes more complex and costly, it is important to effectively assess, as early as possible, the likelihood of adverse effects on non-target organ systems as well. A useful and evolving means to this end is the assessment of phenotypes resulting from genetic inhibition of the gene for an intended target in mice, a species routinely used in pharmacologic and toxicologic testing. Overall genetic inhibition in knockout mouse models allows investigators to look for changes across organ systems to determine which are particularly dependent on the function of the target. In this presentation, an overall screening battery for detecting neurobehavioral and physiological phenotypes will be outlined. Specific examples of phenotypes will be illustrated, including those related to neurologic targets as a means to contribute to the understanding of molecular mechanisms underlying normal and abnormal behavioral processes. In particular, integration of assays that address more complex behaviors such as cognition, sensory and motor function, and affective disposition will be highlighted.

IS 1367 GLOBALLY HARMONIZED SYSTEM OF CLASSIFICATION AND LABELING OF CHEMICALS (GHS): A NEW LANGUAGE FOR TOXICOLOGISTS.

P. A. Weideman[1] and H. D. Burleigh-Flayer[2]. [1]*Schering-Plough Corporation, Union, NJ and* [2]*PPG Industries Inc., Allison Park, PA.*

The United Nations has issued a system for the classification and labeling of chemicals known as the Globally Harmonized System of Classification and Labeling of Chemicals (GHS). The intent of the GHS system is to provide a consistent foundation for classification and labeling of chemicals that takes into account the extensive global trade of chemicals and the need for the development of national programs to deal with the safe supply, use, transport, and disposal of chemicals. This system is being adopted by countries around the world, as well as the newly adopted European chemical legislation, REACH. The challenges that will be faced include the following: 1) available toxicology data used for classification may vary

in quality and consistency between countries and companies, 2) lack of a universal rating system recommended for evaluating the quality of the toxicology data, 3) knowledge and expertise of individuals determining hazard classifications may vary widely, and 4) different countries are not uniformly adopting the GHS, nor the "building blocks" of GHS. Despite the intention of harmonization, it is inevitable that toxicologists will encounter differing classification outcomes for a given chemical because of differences in the availability of information, the evaluation of information, the expertise of the professional (or the regulatory body) performing the evaluation, and differences in implementation of the GHS by various countries or regions. Consistent use by toxicology experts of the criteria and interpretation of data with respect to select endpoints is critical to the success of the GHS and provides for scientific soundness of this hazard communication system. Furthermore, scientists from all disciplines, including those from academia, industry, or government, need to be aware of the GHS and utilize the criteria when applying terminology to chemicals if we are to "speak the same language" and reduce confusion amongst the public and ourselves about chemical hazards.

IS 1368 THE HISTORY, DEVELOPMENT, AND VALUE OF THE GHS.

M. R. Sullivan. *MRS Associates, Arlington, VA.* Sponsor: P. Weideman.

The same chemical product can be classified as toxic in one country and not in another which impacts the protection of workers and consumers, the credibility of industry and professionals and trade. This was recognized in 1992 by the United Nations Conference on Environment and Development (UNCED), also know as the "Earth Summit", which provided the international mandate for the GHS. The GHS work began with the premise that existing systems should be harmonized in order to develop a single, globally harmonized system to address classification of chemicals, labels, and material safety data sheets (MSDS). For over a decade professionals from around the world worked to develop the GHS. Many individuals were involved, from a multitude of countries, international organizations, and stakeholder organizations. Their work spanned a wide range of expertise, from toxicology to fire protection, and ultimately required extensive goodwill and the willingness to compromise, in order to achieve this system. The technical work was divided among three international focal points: the International Labor Organization (ILO); the Organization for Economic Cooperation and Development (OECD); and the United Nations Sub-Committee of Experts on the Transport of Dangerous Goods. Based on their work in the area of testing guidelines and other chemical issues, the OECD was selected as the focal point for health and environmental hazard criteria. This work included chemical substances and mixtures. The GHS is a common and logical approach to defining and classifying the health, physical and environmental hazards of chemicals and to communicating information on labels and MSDS. The GHS "Purple Book" contains guidance on interpreting and implementing the GHS elements. This presentation will cover the basic GHS elements. Chemical products can only have the same hazards globally if professionals and governments embrace and accept the GHS. Global harmonization benefits everyone involved.

IS 1369 GHS IMPLEMENTATION IN THE AMERICAS: ACCEPTANCE AND TIMELINES.

D. A. Deeds. *Industrial Health & Safety Consultants, Inc., Woodbridge, CT.* Sponsor: P. Weideman.

The timing of the adoption of the GHS into the existing Hazard Communication frameworks in the US and Canada has been an issue of much speculation and uncertainty. Now that the EU appears certain to adopt the GHS in 2007-2008 as part of REACH, how will that affect the implementation process in the US and Canada? What sectors plan to adopt the GHS and when and why? Which specific building blocks of the GHS are likely to be adopted? This presentation will attempt to answer some of these questions and will provide an update on progress where the answers are still under development. The presenter will detail the status of GHS adoption in the various sectors (consumer chemicals, the workplace, transportation, pesticides, etc.) in the US, Canada and in other parts of the Americas (Central and South America). Where regulatory changes have already been made to existing systems to facilitate GHS adoption or proposals issued, the impact on existing systems will be identified. Finally, the opportunity for voluntary use of the GHS in these jurisdictions will be discussed.

IS 1370 GHS IMPLEMENTATION IN THE ASIA/PACIFIC RIM: VARIATIONS AND CHALLENGES.

A. M. Johnson. *3M Company, St. Paul, MN.*

Several nations in the Asia/Pacific region have already implemented the use of the GHS through legislative action. The GHS is based on a "building block" approach to classification and labeling; however, implementation of the "building blocks" has been variable among countries in this region. Some of these implementation efforts commenced prior to the finalization of the current GHS guidance which has resulted in significant differences in the interpretation and use of the criteria between the former and current GHS guidance. Additionally, the timing of these implementation efforts will undoubtedly result in changes to the local regulations that have already entered into force, creating more opportunities for differences in labeling and classifications not only in this region, but globally. For example, suppliers and end-users of this "harmonized" system have already been required to meet the multiple national requirements, such as the labeling of mixtures according to the GHS in Japan and the Hazardous Substances and New Organisms (HSNO) classifications of the Environmental Risk Management Authority in New Zealand. Meeting these requirements has already resulted in costly changes in business processes and labeling tools, some of which will require additional modifications to meet updated changes to the GHS in these countries. Other emerging and major markets, including China and Korea, have plans to implement the GHS into other newly implemented chemical management programs in their respective countries. A discussion of the variability and timing of the implementation of the GHS in the Asia/Pacific Rim will be presented.

IS 1371 GHS AND REACH: MEETING THE CHALLENGES OF TWO CHEMICAL MANAGEMENT REQUIREMENTS.

R. Warner. *Chemwise Associates, Oxford, United Kingdom.* Sponsor: P. Weideman.

Implementation of the GHS in Europe is planned to synchronise with the entry into force of the comprehensive chemical legislation in Europe, REACH. The near simultaneous promulgation of these two complex requirements will present very significant logistical and toxicological challenges to chemical manufacturers and users worldwide. The presentation will consider the timing, cost and impact these two new regimes will have on the regulatory landscape in both Europe and the rest of the world. It will include consideration of the requirements for a comprehensive data set (carrying a potential increase in animal testing), and new approaches to risk assessment and risk communication. My thesis is that the particular socio-political imperatives at the time of the negotiation of REACH have led to the introduction of a complex regulatory regime where the science/law architecture lack the rigour which many expected. Furthermore the progressive introduction of GHS (in a similar environment), together with the linkages between GHS and REACH will compound the difficulties to be faced by those who have to meet the practical challenge of compliance. Perhaps against their natural inclinations, many toxicologists are likely to be in the forefront of the inevitable scientific, legal and cultural debates which will have to be held in order to resolve the problems so that the two regimes deliver their promised outcomes. For business, regulatory agencies and professional scientists REACH and GHS will create challenges and opportunities. The essential preliminary to success is understanding the complex motives and forces driving the changes. This presentation will provide a new and stimulating perspective on these important developments.

IS 1372 TOXICOLOGICAL CHALLENGES OF GHS IMPLEMENTATION.

A. R. Schatz. *Global Safety & Environmental Affairs, Schering-Plough Corporation, Union, NJ.*

There are a number of toxicological challenges associated with the implementation of the Globally Harmonized System for classification and labeling of chemicals (GHS). These challenges include: 1) data availability, 2) data quality or reliability, and 3) data interpretation. These issues will result in considerable inconsistency regarding the classification and labeling of chemicals according to GHS. Data transparency is important for GHS; however, confidentiality of company-specific data and the lack of peer-reviewed data-sharing programs are issues. Contributing to this dilemma is the fact that much of the available data are of poor quality, reported conservatively, or not critically reviewed. In addition, most hazard classification tools are predicated on access to electronic data rather than on data quality. The self-classification approach, which allows for and requires significant professional judgment, practically changes GHS to GhS resulting in reduced harmonization. Toxicologists and other professionals responsible for classification and labeling of chemicals, and those who generate data used for classification criteria, must be aware of the differences in GHS implementation in various regions around the world and the implications of "one-stop shopping" for data to support their decisions. A discussion of what the limitations are regarding widely available data sources and guidance to find reliable and peer-reviewed data will be presented. Despite best efforts to make GHS simple and harmonized, professional judgment and expert advice will be required for interpretation of equivocal data, for interpretation of inconsistently reported data, and in situations where significant data gaps exist. GHS is an improvement over the current global condition regarding classification and labeling of chemicals and will go a long way toward harmonization. However, data availability, quality, reliability, and interpretation, remain issues regarding the consistency and harmonization of chemical classifications.

1373 MULTI-MODAL VALIDATION OF COMPUTATIONAL PULMONARY AIRFLOW SIMULATIONS IN THE RAT.

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Accurate computational particle deposition predictions are a necessary step toward linking cell response to site-specific dose and mass transfer in a multiscale model of the rat lung. A phase contrast 3He magnetic resonance (MR) measurement of lobar airflow was acquired in a single live rat. Separately, 1.0-µm fluorescent aerosols were administered to five anesthetized Sprague-Dawley rats. At necropsy, the lungs were air-dried and deposited fluorescent microspheres (FMS) were measured in each of the five rat lobes by fluorescence detection. Finally, computation fluid dynamics models of airflow were developed from three distinct Sprague-Dawley airway casts. Velocity at the trachea was based on ventilator measurements. For the first set of simulations, steady-state inhalation conditions were prescribed, assuming zero-pressure outlet boundary conditions for all airways Subsequently, a transient simulation was performed with the velocity waveform of the ventilator used in the FMS study. 1.0-µm particle deposition simulations were carried out based on both steady-state and transient airflows. The pattern of lobar ventilation distribution measured by FMS was quite reproducible and consistent with 3He MRI data whether or not the animal was on a respirator. Predictions for lobar ventilations based on steady-state assumptions, and with simplified outlet boundary conditions were in excellent agreement with both FMS and 3He MRI measurements, despite inter-animal variability in airway geometry. Transient predictions for lobar ventilations agreed well with FMS results, with minor differences during exhalation. These results represent a first order validation of 3D computational pulmonary airflow simulations in laboratory rodents. Future work includes more detailed time-dependent 3He flow measurements as well as quantitative measurement of localized particle deposition. Funded by NHLBI RO1 HL073598-01.

1374 COMPARING PHARMACOKINETIC MODELS FOR PERFLUOROOCTANOIC ACID IN MICE.

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Perfluorooctanoic acid (PFOA) is environmentally persistent and widely detected in humans and wildlife. Half-lives range from approximately 4 hrs in female rats, to 4 days in male rats, 20–30 days in monkeys, and 3–5 years in humans. Increasingly, reproductive and developmental toxicology studies of PFOA use mice. Hence, single and repeated exposures were used to evaluate PK models in this species. The PFOA single oral dose data included three tissues (blood sera, liver and kidney), two genders, and two doses (1 and 10 mg/kg) in two blocks, collected 4 hrs to 80 days post-dosing. Repeated dose sera data were from adult CD-1 male and female mice exposed to 20 mg/kg for 4 or 17 days. One and two compartment models with first order absorption and elimination rates were fitted using generalized nonlinear least squares analyses in the statistical software R. Model comparisons used a likelihood ratio test. A variant of the saturable reabsorption model of Andersen et al. (Toxicology 227:156-64, 2006) was compared to data using Matlab (Mathworks, Natick MA). The two compartment model converged for only 6 of 24 datasets, and results were not statistically significantly better than for the one compartment model. Model parameters - volume of distribution (Vd), absorption and excretion rates - were determined for the three tissues - serum half-life was 16 and 22 days and Vd 0.135 and 0.226 L/kg in females and males, respectively. Using these values, a one compartment model failed to predict the repeated dose data; a 1.2-day half-life is required for females. The saturable reabsorption model can simulate the single dose and repeated dose data attaining pseudo-steady state in 3–4 days consistent with measured serum levels. PFOA serum concentrations appear linear below 50–100 mg/L, but become increasingly nonlinear above that. This abstract does not necessarily reflect US EPA policy.

1375 PBPK MODELING OF THE MAJOR CHLORPYRIFOS METABOLITE TRICHLOROPYRIDINOL: A POTENTIAL BIOMONITORING STRATEGY.

C. Timchalk, A. L. Busby and T. S. Poet. *Pacific Northwest National Laboratory, Richland, WA.*

Chlorpyfios (CPF) is a commonly utilized organophosphorous insecticide and the major urinary metabolite, trichloropyridinol (TCPy), is used to biomonitor for exposure. Saliva has been proposed as a potential non-invasive biomonitoring matrix, but the utility of saliva TCPy to biomonitor for CPF exposures requires a methodology to correlate saliva TCPy with total CPF dose. In this regard, a TCPy PBPK model was developed to link with a validated CPF model to determine the relationship between blood and salivary TCPy in both rats and humans. The TCPy model includes fat, rapidly perfused, and slowly perfused compartments and compartments from which metabolism of CPF or CPF-oxon leads to TCPy production (brain, diaphragm, liver, and blood). Urinary clearance of TCPy was modeled as first-order elimination from the blood compartment and clearance into the saliva was perfusion-based. The pharmacokinetics of TCP in blood and urine were previously determined in rats following CPF oral doses of 1, 10 and 50 mg/kg, and the results used to calibrate the TCPy model. Based on maximum log likelihood function (MLLF) analysis, the model reasonable fit the TCPy blood and saliva concentrations (82 & 87% variation explained) at all doses with the TCPy concentration in blood exceeded saliva (~2-fold), although the kinetics were comparable. The model also reasonably predicted blood concentrations and urinary elimination of TCPy in humans exposed orally to 0.5 mg/kg CPF dose (MLLF >89% variation explained). These findings suggest that the TCPy PBPK model simulates blood and urinary TCPy concentrations following oral exposure to known doses of CPF in both rats and humans. Secondly based on the rat, the TCPy model simulates the kinetics of TCPy clearance from saliva; suggesting that the model can back estimate CPF dosimetry using saliva TCPy. PBPK modeling of TCPy in saliva may represent a viable quantitative biomonitoring approach with broad application for evaluating both occupational and environmental exposures to CPF. (Supported by CDC/NIOSH grant R01 OH008173-02)

1376 UNCERTAINTY ANALYSIS OF HUMAN EXTRAPOLATIONS BY USE OF A PBPK MODEL FOR 1, 2-DICHLOROETHANE (DCE).

P. M. Schlosser[1] and H. A. Barton[2]. [1]*NCEA, U.S. EPA, Research Triangle Park, NC and* [2]*NCCT, U.S. EPA, Research Triangle Park, NC.*

The U.S. EPA is revising its IRIS assessment for DCE, a high production volume chemical used in production of vinyl chloride and other chlorinated chemicals, as a lead scavenger, fumigant and solvent. DCE exposure causes tumors in multiple tissues of rats and mice. A previous PBPK model for DCE dosimetry in rats (D'Souza et al., 1988) was updated and extrapolated to mice, but no data exist to validate a human version. The model describes oxidation and glutathione (GSH) conjugation of DCE and the oxidative metabolite in liver and lung, along with depletion and replenishment of GSH, and the regulation of GSH synthetase. Thus there are multiple key parameters for which human data are unavailable, resulting in high qualitative uncertainty in the extrapolation. Further, there are 8 dose metrics one can consider. We identified a small number of candidate values for each parameter, covering their likely ranges, yielding 30 possible combinations of these parameter candidates. We then automated the computation of human equivalent exposures (HEEs) for each parameter combination and dose metric, and calculated simple summary statistics for the HEEs for each metric, route of exposure, for both mouse- and rat-based points of departure (PODs). The resulting HEEs varied more than 2 orders of magnitude (across all metrics) and were highly uncertain for a number of specific metrics. But for some metrics the HEEs were quite robust (insensitive) with respect to the parameter options; e.g., DCE AUC varied by a factor of only 1.35 between highest and lowest when extrapolated from the rat inhalation POD. The choice of metric/HEE should also reflect mechanistic considerations (e.g., tissue-specific metabolism is most relevant to toxicity in a tissue) and a desire to be health-protective in the face of remaining uncertainty, but the presentation of results across metrics greatly facilitates selection of the most appropriate.
The analysis, results, and opinions presented are those of the authors and do not necessarily represent EPA policy or decisions.

1377 A PBPK MODELING APPROACH FOR *IN VITRO-IN VIVO* EXTRAPOLATION OF DRUG-DRUG METABOLIC INTERACTIONS.

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In vitro screening assays for drug-drug interactions (DDI) are becoming routine procedures in preclinical research for the selection of drug candidates. However, the interpretation of the generated data for the in vivo situation still represents a challenge. In this study, a mechanistically based modeling approach for extrapolating in vitro metabolic DDI data to the in vivo situation is proposed and applied for binary mixtures of debrisoquine (DBQ), bunitrolol (BUN) and R-bufuralol (BUF) in isolated perfused rat liver (IPRL). The model framework is represented by mathematical descriptions that (i) take into account the physiology of the liver and (ii) enable

integration of distinct drug kinetic processes such as diffusion, partitioning, metabolism and transport processes within the organ. For each binary mixture, single chemical models were pairwisely linked via competition for CYP2D1. Partition coefficients, metabolic rate constants, transport rates and inhibition constants were determined in vitro. The values of these parameters were incorporated into the models to simulate the drugs' perfusate concentration-time profiles in recirculating IPRL when administered alone (2 scenarios each) or in combinations (3 scenarios each). The simulations were compared to corresponding experimentally obtained kinetic data and successfully predicted all 6 sets of single drug data and most interactions data (8 out of 9). Variability may explain the observed discrepancy. This study has successfully demonstrated the potential of the developed model to enable a priori predictions of in vivo metabolic DDIs based solely on in vitro information.

1378 MIXTURES OF AHR FULL AGONISTS, PARTIAL AGONISTS, AND ANTAGONISTS: IMPLICATIONS FOR TEFS.

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Methods to predict the expected outcome of combination exposures are critical both to risk assessment and to an accurate judgment of synergism or antagonism. The Toxic Equivalency Factor (TEF) method used to assess the joint effects of dioxin-like chemicals is a special case of the method of concentration addition. However, the TEF model implicitly assumes that the individual agents are full agonists with parallel dose-response curves. Using kinetic models of simple receptor systems, we derived a generalization of concentration addition (GCA) to describe mixtures of full agonists, partial agonists and competitive antagonists. The GCA and TEF models were assessed for their ability to predict activation of aryl hydrocarbon receptor-dependent gene expression using H1G1.1c3 cells. We tested combinations of TCDD or TCDF with the full agonist PCB 126, the partial agonists PCB 105 and galangin, and the antagonist 3,3'-diindolylmethane (DIM). We compared goodness of fit of the empirical response surfaces to models using the nonparametric Kolmogorov-Smirnov statistic. The GCA and TEF models fit equally well for mixture of full agonists. In the other cases, the GCA model fit the experimental data better than the TEF model. The TEF method clearly overpredicts combination effects at the highest combination doses. At lower (environmentally relevant) doses, the difference between GCA and TEF predictions is dependent on the specific TEF values chosen and the degree of partial agonism, but should be small for many partial agonists, often less than the order of magnitude accuracy that characterizes TEFs. Recent studies have shown that commercial PCB samples can be contaminated with other, more potent congeners, leading to overestimates of both the REP and the maximal effect. As ultrapure PCBs become available for mixtures studies, methods like GCA, which can account for the effect of a low partial agonist on the joint effect of a mixture, will become more important.

1379 BIOLOGY-BASED DOSE-RESPONSE MODELS FOR RISK ASSESSMENT OF CHEMICAL MIXTURES.

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The current orientation in chemicals risk assessment is to tackle them as single substances while, in reality, man is exposed to chemical mixtures. This can result in lower or greater toxicity of mixtures than would be expected from the individual chemicals. The extent of the change in tissue dose depends on the concentrations of all components and the mechanisms of interaction. Herein we present a physiology based pharmacokinetic/dynamic model model for benzene, toluene, ethylbenzene and xylenes that specifically accounts for interactions at the metabolism site. The change in the metabolic rate of each substance is expressed as a function of maximal velocity of metabolism, Michaelis affinity constant, venous blood concentrations of the chemical leaving the site of metabolism, venous blood concentrations of the competing chemicals at the site of metabolism and inhibition constant. We have further elaborated a pathology model to couple the probability of cancer risk to the total concentration of benzene metabolites in the urine across the dose-response space. Extensive validation of the PBPK/PD model against a series of human cohorts corroborated its usability. Our results show that combined exposure modifies risk estimates even at low doses after lifelong exposure to the VOCs mixture. Risk modification is dose-dependent. More important change is found when the VOCs are inhaled at doses that characterize specific working environments (e.g. in refineries or in the paint industry). . Even if all four chemicals are at concentrations that respect the limits set by occupational health regulations, the biologically effective dose of each chemical can be as high as twice the respective dose when it is inhaled individually. Estimation of cancer risk due to benzene requires a detailed calculation of the biologically effective concentrations of its metabolites at the bone marrow. These can be reduced if benzene metabolism is significantly inhibited by the presence of the other three components of the quaternary VOC mix, resulting in lower cancer risk.

1380 MATHEMATICAL MODELING WITH VIRAL CARCINOGENESIS AND ANTI-ONCOGENE KNOCKOUT MICE.

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In multicellular organism, cells with genomic damages have multiple changes to escape the check-point-arrests for apoptosis or oncogenic senescence. This has been shown in the experimental animals with genotoxic chemical exposure. Using the Weibull's time-to-tumor mathematical model, we show that cancer develops from the multiplicative probabilities from
Losing the functions of p53/p21/H2AX in antioncogenes-knockout mice-colonies earlier and in Maloney murine-leukemia-virus (MMLV) infected mice now. Viruses such as MMLV or Human-T-cell-Leukemia-Virus (HTLV) or Kaposi sarcoma virus can integrate and recombine with the host genome to affect those antioncogenes functions controlling the cell cycle bringing about cancer synergistically. This is demonstrated mathematically in the current effort and cytogenetically in our previous publications. The variability of integrative sites has been shown in MMLV, 5' upstream or downstream to the promoter affecting different antioncogenes. This variation in integration sites bringing about various cancer developments
gives a concrete meaning of multipath in carcinogenesis as the various path of cell cycle control. Moreover, the multistage of cancer progression at the organismic level as first defined by Leslie Foulds can now be related to gene modifications (such as p53, p21, Arf) at the cellular level. The control of and the path (multipath) of carcinogenesis is the combination of different antioncogene regulating cell proliferation. Apoptosis and Senescence can now be equated to the initiation and promotion event followed by the progression process. Weibull model fits to time to tumor data indicated a 6th power relationship; this is consistent with current understanding of the multiple steps in viral carcinogenesis as discussed above."

Disclaimer: The opinions and conclusions in this abstract are only those of the authors and do not reflect the institutions they represent.

1381 DEVELOPMENT OF A BIOLOGICALLY BASED DOSE RESPONSE (BBDR) MODEL FOR ARSENIC INDUCED CANCER.

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The current USEPA cancer risk assessment for inorganic arsenic uses linear extrapolation of the epidemiological data from exposed populations in Taiwan. However, proposed key events in the mode of action for arsenic-induced cancer (which may include altered DNA methylation, altered DNA repair, and induced reactive oxygen species) suggest the possibility of a nonlinear dose-response at low doses. We are developing a BBDR model for arsenic carcinogenicity in order to reduce uncertainty in estimates of low dose risk, whether or not the dose-response is nonlinear, by maximizing the use of relevant data on the mode of action. Expert consultation and literature review are being conducted to identify the tumor endpoints of regulatory interest and the sequence(s) of key events most likely to be linked to cancer. Laboratory studies will be designed to emphasize dose-response and time course behaviors for the key events. This type of experimental design is necessary if the data are to fully support the BBDR model. The level of biological detail in the model will be determined largely by the availability of data – i.e., model structure will not include large amounts of detail that cannot be parameterized against data. The initial modeling effort will include a description of the pathway leading from DNA damage (subsequent, for example, to As-induced oxidative stress) to activation of cell cycle checkpoints and apoptosis. The full BBDR model will consist of (1) a physiologically-based pharmacokinetic (PBPK) submodel to provide estimates of tissue-specific doses, (2) the sequence(s) of key events (mode of action) triggered by the tissue dose and (3), a multistage clonal growth model to predict dose-responses and time-courses for tumor incidence. This coordinated data collection – model development effort will increase our understanding of how biological factors determine the shape of the dose-response curves for arsenic-induced cancer. *This abstract has been reviewed by EPA and approved for publication but does not necessarily represent Agency policy.*

1382 OVOTOXICITY OF 4-VINYLCYCLOHEXENE DIEPOXIDE MEDIATED THROUGH PHOSPHATIDYLINOSITOL 3-KINASE SIGNALING.

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4-Vinylcyclohexene diepoxide (VCD) is an occupational chemical that destroys ovarian small pre-antral follicles in mice and rats. A post-natal day 4 (PND4) rat whole ovary culture system (enriched in target small pre-antral follicles) has been used in mechanistic studies to investigate ovotoxic effects of VCD. Previous studies have shown that c-kit signaling pathway members are inhibited by VCD exposure. One member of that pathway for oocyte survival is phosphatidylinositol 3-kinase (PI3 kinase). The present study was designed to investigate the involvement of PI3 kinase in VCD-induced ovotoxicity. Ovaries from PND4 female fisher 344 rats were cultured for 8 days in control media or VCD (30μM) ± PI3 kinase inhibitor, LY294002 (10μM and 20μM). Following culture, ovaries were histologically evaluated by follicle classification and counting. Relative to control, VCD caused loss ($p<0.05$) of primordial (control = 949 ± 149; VCD = 152 ± 52, follicles counted) and primary follicles (control = 64 ± 22; VCD = 21 ± 12, follicles counted). There was no effect of LY294002 alone (10μM or 20μM) on follicle viability or primordial follicle number. Interestingly, at 20μM LY294002, follicle numbers were reduced ($p<0.05$; primary to 17% of control and secondary to 6% of control). In incubations with LY294002 (20μM) plus VCD, primordial follicle numbers were not different ($p>0.05$) from control (control + LY294002 = 858 ± 201; VCD + LY294002 = 717 ± 39, follicles counted). These results demonstrate that 1) LY294002 attenuated VCD-induced follicle loss and 2) LY294002 prevented primary and secondary follicle recruitment. These findings support that both VCD-induced ovotoxicity and pre-antral follicle recruitment in the rat ovary are mediated through a PI3 kinase signaling pathway. These findings further support that VCD-induced ovotoxicity may interact directly with the recruitment pathway from primordial to primary follicle (Supported by ES09246 and Center grant 06694).

1383 OOCYTE INJURY CAN BE DETECTED AFTER *IN VITRO* EXPOSURE TO BI-FUNCTIONAL TOXICANTS.

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Mammalian oocytes are a unique cell type arrested in meiosis in adults. The defence mechanisms and fate of these cells following DNA damage is unknown. They may be repaired, lost, or remain to produce offspring. Phosphoramide mustard (PM) is the active metabolite of the chemotherapeutic drug cyclophosphamide and is a model DNA-damaging chemical. A neonatal ovarian culture system was used to determine the effect of PM on primordial follicle oocytes. Post-natal day 4 (PND4) ovaries from CD-1 mice or Sprague-Dawley rats were cultured in PM (0.1-10μM and 1-100μM, respectively). Phosphorylated histone H2AX (γ-H2AX) foci were examined as a marker of DNA double-strand breaks. Maximum numbers of γ-H2AX foci were observed in oocytes of primordial follicles from CD-1 mice after 18-24 hours of PM exposure. In both species, PM (0.3-1μM and 3-10μM; below concentrations which produce follicle loss) resulted in increased numbers of follicles with ≥5 γ-H2AX foci/oocyte. 4-vinylcyclohexene diepoxide (VCD) is an occupational chemical that destroys ovarian small pre-antral follicles in rats and mice. VCD is similar to PM in that both are bi-functional toxicants with two reactive groups, but the effects of VCD on DNA are unclear. Exposure of neonatal mouse ovaries in vitro to VCD (≥20μM for 2 days) induces follicle loss ($p<0.05$). VCD treatment caused γ-H2AX foci formation in primordial follicle oocytes 4 days after exposure to VCD (10 or 30μM for 2 days). This suggests that the mechanism of VCD-induced primordial follicle loss may involve DNA damage. These results suggest that in spite of species-specific differences in sensitivity to PM and VCD between mice and rats, primordial follicle oocytes suffer DNA damage when exposed to these two bi-functional toxicants. Additionally, these results support that the mechanisms of ovarian toxicity induced by PM and VCD may be similar [This study was supported by NSERC].

1384 SEXUAL MATURITY-RELATED CHANGES IN OVARIAN FOLLICLE GROWTH IN ARYL HYDROCARBON RECEPTOR-DEFICIENT MICE.

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The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor in the cytosol of most cells, including those in ovarian follicles. Toxicants such as polychlorinated biphenyls and dioxins bind to the AHR and lead to toxic effects. Studies have shown that deletion of *Ahr* gene affects fertility and ovarian follicle numbers. In this study, we compared the ovarian follicular growth of AHR-deficient (AHRKO) and wild-type (WT) mice at different ages of sexual maturity. Antral follicles were isolated and cultured from AHRKO and WT mice on postnatal days (PD) 30 (prior to puberty), PD 54 (recent-cycling adults) and PD 90 (cycling adults). Growth in culture was evaluated for 168 h by measuring the diameters of follicles in two perpendicular axes. The diameters of WT and AHRKO follicles from PD 30 were similar at the beginning of culture (WT=338±13 μm; AHRKO=329±9 μm; $p=0.08$), but AHRKO follicles had a smaller diameter than WT follicles by 168 h (WT=684±18.4 μm; AHRKO=537±11.6 μm; $p<0.001$). Interestingly, differences in growth may be observed in follicles collected from mice at PD 54 (WT=594±16.3 μm; AHRKO=550±12.7 μm; $p=0.056$), but not in follicles at PD 90 (WT=531±17.2 μm; AHRKO=530±18.4 μm; $p=0.094$). Further, the number of follicles that reached the preantral and antral stages in vivo was histologically evaluated. At PD 30, WT and AHRKO ovaries had similar numbers of preantral and antral follicles. By PD 54, however, AHRKO ovaries had fewer preantral and antral follicles than WT ovaries ($p<0.05$). Interestingly, by PD 90, WT and AHRKO ovaries contained similar numbers of preantral (WT=170±28; AHRKO=164±24; $p=0.97$) and antral follicles (WT=167±20; AHRKO=160±25; $p=0.56$). These results suggest that the AHR regulates follicular growth before puberty, but may not regulate it after puberty. Although the reasons are unknown, the differences observed at different ages might be the result of alterations in hormone levels and their corresponding receptors, and/or factors involved in the translocation of the AHR in the cells. Support: NIH HD047275.

1385 OVEREXPRESSION OF GLUTAMATE CYSTEINE LIGASE PROTECTS GRANULOSA CELLS AGAINST OXIDANT AND RADIATION-INDUCED CELL DEATH.

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We have shown that depletion of the antioxidant glutathione (GSH) sensitizes ovarian follicles and granulosa cells to toxicant-induced apoptosis and that supplementation of GSH reverses this effect. The first and rate-limiting reaction in GSH synthesis is catalyzed by glutamate-cysteine ligase (GCL), which is comprised of a catalytic (GCLc) and a regulatory subunit (GCLm). We tested the hypothesis that overexpression of Gcl protects granulosa cells against oxidative stress- and radiation-induce cell death. The COV434 line of human granulosa cells was stably transfected with a vector designed for the human cytomegalovirus immediate early promoter-mediated constitutive expression of Gclc (GCLc-transfected), Gclm (GCLm-transfected), Gclc and Gclm (GCL-transfected), or empty vector (control). Mean GCL protein levels and total GSH levels were significantly increased in GCL-subunit transfected cells. Cells were treated with 0 to 10 mM hydrogen peroxide and then assayed for cell viability. GCLc-transfected cells had significantly higher numbers of viable cells after hydrogen peroxide treatment than control cells. Cells were treated with 0.5 mM hydrogen peroxide for 0 to 8 hours and then similarly assayed. Cell viability in all of the GCL subunit transfected cell lines was significantly higher at all times after hydrogen peroxide than in the control cells. Cells were irradiated with 0, 1, or 5 Gy of gamma rays and cell viability was assayed after 2, 3, and 4 days. GCLc-transfected cells were protected against radiation-induced cell death. At the 5 Gy dose GCLc-transfected cells showed an increase in viable cells with time whereas the control cells did not proliferate. These findings suggest that granulosa cells overexpressing GCL, due to their increased ability to synthesize GSH, are able to respond to and overcome the oxidant and radiation insults. Supported by NIH ES10963.

1386 OVEREXPRESSION OF ESTROGEN RECEPTOR ALPHA INCREASES SENSITIVITY TO METHOXYCHLOR IN THE MOUSE OVARY.

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During development, normal levels of estrogen are critical for the proper development of various systems including the reproductive system. Chemicals such as the organochlorine pesticide methoxychlor (MXC) may disrupt normal functions of estrogen by either obstructing its normal course of action or interfering in the physiological response to the hormone. To examine whether MXC causes atresia of antral follicles through an estrogen receptor alpha (ESR1) pathway, we have generated and validated a transgenic mouse model in which ESR1 is overexpressed in several tissues (ESR1-OE), including the ovaries. In addition, this overexpression can be turned off in the presence of doxycycline. To determine whether ESR1 overexpression affects the ability of mice to respond to MXC, controls and ESR1-OE mice were treated with either vehicle or MXC (32 and 64 mg/kg/day) for 20 days. The ovaries of these mice were collected and subjected to histological evaluation of

the percentage of atretic follicles (PAF) and the thecal cell thickness (TCT) of antral follicles. The results indicate that the PAF was significantly increased in ESR1-OE and control mice treated with MXC compared to sesame oil (S) controls. Interestingly, ESR1-OE mice treated with MXC had a significantly higher percentage of atretic follicles than controls treated with MXC (controls/S = 9.18%, 32 mg/kg/day MXC = 19.53%, 64 mg/kg/day MXC = 21.58%; ESR1-OE/S = 9.56%, 32 mg/kg/day MXC = 19.5%; 64 mg/kg/day MXC = 37.23%; n = 3; $p \leq 0.05$). TCT was found to be significantly less in ESR1-OE mice treated with MXC than TCT in ESR1-OE mice treated with S (S = 5.95 µm ± 0.11, 32mg/kg/day MXC = 5.35 µm ± 0.6, 64mg/kg/day MXC = 5.06 µm ± 1.02; n = 3, $p \leq 0.05$). Further TCT was significantly less in ESR1-OE mice treated with 64mg/kg/day MXC compared to control mice treated with 64mg/kg/day MXC (controls = 5.97 µm ± 1.2; ESR1-OE = 5.06 µm ± 1.02; n = 3, $p \leq 0.05$). Collectively, these results indicate that ESR1 overexpression in this novel transgenic mouse model increases the sensitivity of the ovaries to MXC. Support: NIH R21ES13061, RO1ES012893.

1387 CONCENTRATION DEPENDENT EFFECTS OF GENISTEIN IN NOVEL MODELS OF ESTROGEN-STIMULATED ENDOMETRIUM.

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Estrogen is the leading etiologic factor in the development of endometrial cancer, although the mechanism of estrogen-induced carcinogenesis is unclear. Genistein, a phytoestrogen found in soy, is known to bind the estrogen receptors (ER) and therefore partially mimic the effects of estrogen. Although research has demonstrated the health benefits of a soy-rich diet, nutritional studies of genistein remain contradictory. A novel endometrial co-culture model that reconstitutes the heterotypic cell-cell interactions observed in vivo was used to characterize estrogen-induced proliferation and the concentration-dependent effects of genistein in this model. Estrogen-stimulated proliferation of the endometrial epithelial cell (EEC) component of our model was shown to require expression of soluble insulin-like growth factor (IGF). A correlation between increasing genistein and increasing EEC estrogen receptor activity was observed, although no change in EEC proliferation occurred with genistein alone. However, co-cultures treated with both estrogen and genistein demonstrated that genistein modulates estrogen-driven EEC proliferation in a biphasic manner. Low concentration genistein antagonized estrogen-driven EEC proliferation, whereas high-concentration genistein increased the estrogenic effect. Protein arrays and immunoblot analysis of soluble factors affected by estrogen and genistein showed a correlation between changes in EEC proliferation and changes in IGF-1 pathway components, where inhibitory genistein counteracted the effects of estrogen alone and estrogen-promoting concentrations of genistein enhanced the estrogen-dependent changes in the IGF-axis. These novel data demonstrate the IGF-dependent biphasic response of estrogen-stimulated endometrial co-cultures to genistein, and suggests a threshold concentration of genistein above which genistein becomes an estrogenic, potentially carcinogenic compound.

1388 *IN VIVO* METABOLITES OF DE-71 ARE ESTROGENIC *IN VITRO*.

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Polybrominated diphenyl ethers (PBDEs) are widely found in the environment and they are suspected to act as endocrine disruptors. Previous research in our laoratory show that DE-71 is a weak estrogen in the MCF-7 breast cancer cell prolieration model and in ovariectomized BALB/c mice. We tested the PBDE mixture DE-71 and its in vivo metabolites found in mice for their ability to activate Estrogen Receptor Alpha (ERα). Estrogen Response Element (ERE)-luciferase gene expression and 3H-β -estradiol (3H-E2) displacement from recombinant ERα served as estrogen bioassays. Although DE-71 did not bind ERα, it was able to induce ERE-luciferase expression. Six OH-PBDE metabolites were found in mice after 34-day treatment with DE-71 and all were able to
displace 3H-E2 from recombinant ERα. The para-hydroxylated metabolites 4'-OH-BDE-17, 4-OH-BDE-42, and 4'-OH-BDE-49 displayed a 10- to 30-fold higher affinity for ERα
compared to the ortho-hydroxylated PBDEs. Three para-OH-PBDEs induced ERE-luciferase. Although it was 3,000-fold less potent than β-estradiol, 4'-OH-BDE-17 was 10-fold more potent than DE-71 and its maximal effect was 30% higher than that produced by β-estradiol. DE-71 and some of the para OH-PBDEs produced an additive effect of when coadministered with β-estradiol. At high concentrations (≥ 5x10-5 M), the ortho-OH-PBDEs, 2-OH-BDE-28 and 6-OH-BDE-47, were antiestrogenic in the ERE-luciferase assay. The observations that the DE-71 mixture does not displace 3H-E2 from ERα while the hydroxylated metabolites do, suggest that the cellular and tissue effects of DE-71 were due to a metabolic activation of individual congeners. [This research was supported by a grant from NIEHS, F31ES013341]

1389 EFFECTS OF NUTRITION RELEVANT MIXTURES OF PHYTOESTROGENS ON STEROIDOGENESIS AND ON AROMATASE, ESTROGEN, AND ANDROGEN ACTIVITIES.

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Sponsor: L. Gray.

Phytoestrogens (PEs) are naturally occurring plant components. They can mimic or modulate the action or production of endogenous hormones. The broad classes of PEs include isoflavonoids, lignans and coumestans. This study was designed to examine mixtures of twelve PEs for effects on: 1) steroid hormone production in human adrenal corticalcarcinoma cells (H295R) 2) aromatase activity in human JEG-3 choriocarcinoma cells 3) estrogenic activity as determined by proliferation of human MCF-7 cells and 4) androgen receptor agonism/antagonism as determined in a reporter gene assay. The selection of PEs (genistein, daidzein, equol, formononetin, biochanin a, enterolactone, enterodiol, coumestrol, secoisolarici-, matai-, larici- and pinoresinol) was based on the intake of a number of PEs in the Swedish population, and tested at human relevant doses within the range 100pM to 10µM. The selected PEs were tested either in subgroups or in a mixture of all compounds containing equimolar concentrations. Our results showed that all PEs tested were able to disturb steroidogenesis in H295R cells resulting in an increased estradiol production. Additionally, several PEs caused a reduction in the testosterone level. Overall, these results indicate that a stimulation of aromatase activity is taking place. This was confirmed in the JEG-3 aromastase assay in which all PEs (except daidzein and equol) induced the aromatase activity. Furthermore it was shown that PEs belonging to the group of isoflavonoids and coumestans resulted in stimulation of MCF-7 proliferation and that none of the selected PEs affected the androgen receptor. The most noticeable mixture effects were seen in the H295R assay in which lower doses of all twelve PEs caused a marked effect on estradiol production compared to the effects of single PEs/subgroups.

1390 A TRIPLE BIOFLAVONOID MIXTURE MODULATES PRO- AND ANTI-APOPTOTIC GENE EXPRESSION DURING STREPTOZOCIN (STZ)-INDUCED OXIDATIVE STRESS IN THE MOUSE LIVER.

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Lack of hepatic control of glucose metabolism and insulin insensitivity are considered instrumental to hyperglycemia ultimately resulting in diabetes. Despite the availability of diverse antidiabetic agents, numerous epidemiological studies have correlated human consumption of select diets rich in fruits and vegetables containing high levels of phytochemicals to lower risk of diabetes. It is believed that, during hyperglycemia, both pancreas and the liver remain under continuous oxidative challenge. Therefore, this study was designed to investigate whether a mixture of bioflavonoids can: i) potentiate antioxidant pathways, ii) reduce hyperglycemic condition, and iii) minimize apoptotic/necrotic cell deaths in the liver of STZ-induced diabetic mice. Three mechanistically sound powerful antioxidant phytochemicals- rutin, hesperidin and curcumin were chosen for this study. Diabetes was induced in 6 mos old female ICR mice by STZ (100 mg/Kg, ip) administration, and the bioflavonoid mixture was orally administered to diabetic mice for 14 days (blood glucose levels were >+250 mg/dl). Treatment with the bioflavonoids considerably reduced blood glucose, prevented rapid loss of body weight and mortality in diabetic mice. Flavonoid exposure caused a significant drop in oxidative stress (STZ: 21.6 and STZ+Flavonoid: 12.8 nmol MDA/g. liver), total nitrate/nitrite levels and a dramatic increase in antioxidant profile (SOD, catalase and GSH) in the livers. Results show STZ induced down-regulation of anti-apoptotic genes (BcL-2, and BcL-xL) and up-regulation of the pro-apoptotic-Cyt C was reversed by the bioflavonoids, and this effect was mirrored in incidence of apoptotic/necrotic cell deaths. In conclusion, this study for the first time may have shown, modulation of hepatic pro- and anti-apoptotic genes during diabetes, and also demonstrated that a mixture of bioflavonoids may potentially hinder several deleterious STZ-induced consequences in the liver.

1391 EFFECT OF SPECIFIC GENE SILENCING ON SDZ IMM125-INDUCED CASPASE-3 AND -7 ACTIVATION IN CULTURED RAT HEPATOCYTES.

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SDZ IMM 125 is a cyclosporin-derivative known to activate caspase-3/7 in cultured rat hepatocytes. Co-incubations with a caspase-3/7-inhibitor (Ac-DEVD-CHO) led to reduction of caspase-activation and showed beneficial effects on cell survival in previously published data. Since the specificity of the applied caspase-inhibitor is overlapping for caspase-3, -7 and maybe also with other caspases, the highly specific siRNA approach was used to specifically knock-down the activity of caspase-3.siRNAs were delivered by electroporation to rat primary hepatocytes isolated by collagenase perfusion. Cells were kept in culture for 96 h after electroporation to allow the target-protein to deplete, then activated by 4-5 hour incubation with SDZ IMM 125. Caspase-3/7-activity and lactate-dehydrogenase-leakage to the culture supernatant were investigated as endpoints. Caspase-3/7-activity became evident 2 h after substance-treatment, followed by LDH-release after 4.5 h. Specific knock-down of caspase-3 lead to strong reduction of caspase-3/7-activation by 77 ± 10 % of the enzymatic activity compared to the background activity in cell culture. Cells transfected with siRNA against caspase-7, did not show decreased caspase-3/7 activity after SDZ IMM125 treatment.
The effects of the caspase-3/7-inhibitor was adjusted by concentration-dependent dilution. At a concentration of 5 μM Ac-DEVD-CHO, the decrease of the caspase-3/7-activity was equal to that achieved by siRNA. At this concentration, no protection against SDZ IMM125-induced cytotoxicity was evident. However relevant cytoprotection was achieved by 75 μM Ac-DEVD-CHO, at which caspase-3/-7 activities were about 50 % of activities of that in untreated control cells. It is very likely that the SDZ IMM125-induced caspase-3/-7 activation is specifically mediated via caspae-3 and not caspase-7. The caspase-3 activity threshold for achieving cytoprotection was determined to be 50 % of the normal control cell activity.

1392 MODIFIED CARDIOLIPIN HOMOLOGUES AS INHIBITORS OF PRO-APOPTOTIC PEROXIDASE FUNCTION OF CYTOCHROME C.

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Upon complex formation with unsaturated cardiolipins, cytochrome c (cyt c) turns from a mitochondrial electron carrier into a peroxidase which can oxidize small reducing substrates, protein tyrosines and cardiolipin (CL) itself. Cyt c-driven CL oxidation appears to be one of the key events at the initial stage of mitochondria-dependent apoptosis triggered by different toxic chemical agents. We found that tetraoleoyl CL (TOCL), in which one of its four acyl chains was modified by a covalent attachment of fluorescent NBD moiety (NBD-CL) – also effectively formed complexes with cyt c. In contrast to cyt c/CL complexes, cyt c/NBD-CL did not induce oxidation of typical peroxidase substrates (etoposide, amplex red). Characteristic formation of Tyr-Tyr crosslinks and aggregation of cyt c was also significantly inhibited. Kinetic studies demonstrated that inhibition of cyt c/NBD-CL peroxidase activity occurred via reversible mixed mechanism. We were able to detect H2O2-dependent decay of NBD fluorescence in cyt c/NBD-CL complex, likely due to oxidation of NBD. By using ESI-MS analysis we found that a new product with a lower molecular mass was formed from NBD-CL. This suggests that the NBD moiety acted a substrate for cyt c in its complex with NBD-CL; NBD donated an electron to highly oxidized state of heme and outcompeted other substrates. Most importantly, NBD-CL prevented cyt c-dependent oxidation of TLCL and formation of hydroperoxides – oxidized CL species essential for the release of pro-apoptotic proteins from mitochondria. We conclude that synthesis of new CL homologues capable to bind cyt c and block its peroxidase activity may be a promising approach for design of new anti-apoptotic agents. Supported by NIH 1R03TW007320-01A1, NIH U19 A1068021.

1393 STRUCTURE ACTIVITY OF METHOXY SUBSTITUTED PHENOLS: CYTOTOXICITY TOWARD ESTROGEN RECEPTOR NEGATIVE HUMAN BREAST CANCER CELLS.

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Estrogen receptor α negative (ERα-) breast cancer is an aggressive form of breast cancer that requires effective drug treatment and thus, the search for a novel drug therapy continues. Certain polyphenolic compounds have become a focal point in cancer research and structure activity relationship studies have shed light on the structural characteristics of these compounds that contribute to their anti-cancer properties. We hypothesized that the presence of a methoxy functional group on a phenolic ring is key to cytotoxicity. Therefore, this study assessed the cytotoxic effects of 6 different methoxy substituted phenols (namely, 7,8-dimethoxyflavone, 4-methoxy benzophenone, 4-methoxy phenylacetic acid, 2-methoxy phenylacetic acid, 5-methoxy-1-indanon and coenzyme Q0 (CoQ0)) in 2 ERα- human breast cancer cell lines (MDA-MB-231 and SkBR3). The results from sulforhodamine B assays demonstrated that CoQ0 was significantly cytotoxic in both cell lines. Specifically, 5 days of treatment with CoQ0 (7.5 μM) caused 100% cytotoxicity in MDA-MB-231 cells and decreased SkBR3 cell number by 84 ± 2% of control. Of the other compounds examined, only 7,8-dimethoxyflavone elicited cytotoxicity in both cell lines (cell number reduced by 70 and 60% for SKBR3 and MDA-MB-231 cells, respectively). Therefore changes in cell cycle progression and induction of apoptosis as mechanism(s) for cytotoxicity were examined following treatment with CoQ0. Cell cycle progression, using propidium iodide staining and flow cytometry, revealed that 24 hr of treatment with 7.5 μM CoQ0 increased the proportion of cells in G1/G0-phase by 16.6 ± 0.6% of control. Additionally apoptosis examination, using annexin V and propidium iodide staining, demonstrated that 24 hr of 7.5 μM of CoQ0 increased the number of apoptotic cells by approximately 11-fold over control. In conclusion, our initial screen has identified CoQ0 as a potent cytotoxic drug that induces apoptosis in ERα- breast cancer cells. Therefore, this compound is an appropriate candidate for further study as a drug for ERα- breast cancer.

1394 A NUTRIENT MIXTURE INDUCES APOPTOSIS IN HUMAN RENAL CELL CARCINOMA 786-0 AND HUMAN MELANOMA A2058.

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Renal cell carcinoma (RCC) is erratic and unpredictable even when diagnosed. The incidence rates for RCC are higher among African Americans and the male to female ratio for RCC is 2:1. At diagnosis, five-year survival is limited to 60% in RCC patients. Melanoma, a very serious form of skin cancer, is a relatively rare cancer. However, its incidence rate in the U.S. has been increasing steadily, and it was the sixth most common cancer in the U.S. in 2003. Though often curable in its early stages, melanoma can metastasize to other areas of the body. We have characterized in our laboratory a nutrient mixture (NM) containing lysine, proline, ascorbic acid and green tea extract as a novel antineoplastic agent with a broad spectrum of antitumor activity against a number of human cancer cell lines. We investigated whether the underlying antitumor effect of NM observed in human RCC and melanoma was due to apoptosis. Human RCC 786-0 and human melanoma cells A2058 (ATCC) were cultured in RPMI and DMEM medium respectively and treated with NM in different concentrations: 0, 100, 500 and 1000 μg/ml. Growth inhibition was detected by MTT assay, morphology by H&E staining, and apoptosis by Live Green Caspase Detection Kit. NM showed no significant effect on RCC growth, exhibiting slight toxicity at 100 μg and significant at 500 and 1000 μg/ml. RCC 786-0 cells treated at 500 and 1000 μg/ml NM and melanoma A2058 cells treated with 100, 500 and 1000 μg/ml NM and stained by H&E demonstrated obvious apoptotic cells with shrinkage and darkly stained and condensed nuclei and strong acidophilic cytoplasm. Using live green caspases kit a significant number of early and late apoptotic RCC cells were demonstrated at 500 and 1000 μg/ml NM and a considerable number of apoptotic melanoma cells at 100 μg/ml, with significant increase at 500 and 1000 μg/ml NM. Our results suggest that the anticancer activity of NM on RCC and melanoma cells is due in part to its apoptotic effect on these cell lines.

1395 ASPIRIN-INDUCED CELL CYCLE ARREST, MITOCHONDRIAL DYSFUNCTION, OXIDATIVE STRESS AND APOPTOSIS IN HEPATOMA HEPG2 CELLS.

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The anticancer and chemopreventive effect of aspirin (acetylsalicylic acid) has been shown to be partly independent of COX inhibition. Multiple pathways, including cell cycle dysregulation, alterations in signal transduction and apoptosis are presumed to be involved. The precise molecular mechanisms, however, are not well understood. We, therefore, have investigated the dose- and time- dependent effects of aspirin using multiple molecular targets, including mitochondrial functions, cell cycle regulation, oxidative stress, glutathione (GSH) homeostasis and apoptosis in HepG2 cells. Our results have demonstrated cell cycle arrest in the Go/G1 phase, an increase in reactive oxygen species (ROS), lipid peroxidation, loss of mitochondrial membrane potential and ATP production, accompanied by inhibition of the

activities of the respiratory enzymes, aconitase, NADH dehydrogenase and cytochrome c oxidase. The treated HepG2 cells undergo apoptosis triggered by the release of cytochrome c from the mitochondria and activation of caspases. In addition, the expression of antiapoptotic protein Bcl2, as well as iNOS and COX-2, was also inhibited in aspirin treated cells. Furthermore, we have also demonstrated that the GSH pool and the activities of the GSH-metabolising enzymes, glutathione S-transferase and GSH-peroxidase were increased and GSH-reductase was decreased in these cells. Some of the key mitochondrial functions affected by aspirin appear to be associated with the alterations in GSH pool as buthionine sulfoximine (BSO), a GSH depleting agent, treatment has augmented its effects and the supplementation of N-acetylcysteine has attenuated the effects of BSO. Our results strongly suggest that the anticancer effects of aspirin on cancer cells might be associated with mitochondrial dysfunction and oxidative stress.

1396 CARBACHOL PREVENTS OXIDATIVE STRESS-MEDIATED APOPTOSIS INDUCED BY DOMOIC ACID IN CEREBELLAR GRANULE CELLS.

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In mouse cerebellar granule neurons (CGNs) low concentration of domoic acid induce apoptotic neuronal cell death, which involves caspase-3 activation and is mediated by oxidative stress. The cholinergic agonist carbachol caused a concentration-dependent inhibition of domoic acid-induced apoptosis, which was antagonized by atropine and by the M3 muscarinic receptor antagonist 4-diphenylacetoxyl-N-methylpiperidine methiodide. Carbachol also inhibited domoic-acid-induced activation of caspase-3 and of p38 and JNK kinases. Carbachol did not prevent domoic acid-induced elevation in ROS, suggesting that the antiapoptotic effect of carbachol occurs downstream of ROS production. Carbachol induced phosphorylation of Akt and of Erk 1/2, without altering the level of expression of unphosphorylated protein.
The protective effect of carbachol was reversed by specific inhibitors of pro-survival intracellular signaling proteins, including an inhibitor of phosphatidylinositol 3-kinase (LY294002), of Akt (Akt inhibitor III) and of MEK/MAPK (UO126).
It has been shown that activation of the PI3K/Akt pathway can prevent oxidative stress-mediated apoptosis through the transcription-dependent elevation in glutamate-cysteine ligase (GCL) activity, which is the rate limiting step in the synthesis of glutathione (GSH). Carbachol caused a time –dependent increase of GCL activity and of GSH levels. Both effects were antagonized by inhibitors of the PI3K/Akt pathway, but not by the inhibitor of the MEK/MAPK pathway. This increase in GCL activity occurred without any change in the level of expression of the catalytic and modifier subunits. These results indicate that stimulation of muscarinic receptor activates the PI3K/Akt pathway leading to increase of GCL activity. The resultant increase in intracellular GSH levels restores the cellular redox balance antagonizing the oxidative stress-mediated apoptosis induced by domoic acid. Supported by ES012762/NSF-OCE-0434087.

1397 THE EFFECTS OF JS-K AND PABA/NO, NOVEL ANTI-TUMOR NITRIC OXIDE RELEASING PRODRUGS, IN HUMAN HEPATOMA HEPB3 CELLS.

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The O^2-arylated diazeniumdiolates JS-K and PABA/NO are nitric oxide (NO) releasing prodrugs that have been shown to have anticancer activity in a variety of model systems, including human HL-60 leukemia cells, multiple myeloma cells, prostate cancer PPC-1 cells, ovarian A2780 cancer cells, and JM-1 hepatoma cells. To expand these findings, the present study examined effects of JS-K and PABA/NO in human hepatoma HepB3 cells. HepB3 cells were treated with various concentrations of JS-K or PABA/NO, and cytolethality was determined 48 hrs later by the MTS assay. The LC_{50} values for JS-K was about 15 μM, and for PABA/NO 20 μM, while the specific chemical compound controls lacking the NO moiety (JS-43-126 for JS-K and JS-39-93 for PABA/NO) showed no apparent cytotolethality at levels up to 100 μM. At well tolerated concentrations (3-10 μM), both JS-K and PABA/NO potentiated the cytolethality produced by sodium arsenite, an emerging chemotherapeutic metalloid, and the LC_{50} for arsenite was shifted from 20 μM to about 10 μM. To examine gene expression changes associated with JS-K and PABA/NO induced tumor cell death, HepB3 cells were treated with 0-10 μM JS-K or PABA/NO for various times (12 - 48 hrs), and total RNA was isolated, purified, and subjected to real-time RT-PCR analysis. JS-K and PABA/NO treatment induced changes in gene expression typical for cell death and growth arrest. These included increased expression of genes associated with apoptosis (*caspase 3* and *caspase 8*), growth arrest (*p53*), acute phase response (*c-jun* and *IL-1β*), anti-angiogenesis such as ribonuclease/angiogenin inhibitor, *RA1*. Both NO releasing compounds also altered various genes related to tumor cell invasion such as tissue inhibitors of metalloproteinases (*TIMP1* and *TIMP2*). Thus, multiple molecular events are likely associated with JS-K and PABA/NO modulated cell death in human HepB3 hepatoma cells, and may contribute to an overall antitumor effect.

1398 GOLD NANOPARTICLES IN RATS AFTER INHALATION EXPOSURE.

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Gold nanoparticles (nAu) are used in consumer products, suggesting potential exposure to this material during manufacturing process or use of consumer products. Since gold in bulk form is chemically inert compared to other metallic materials, any biologic effects of nAu are likely due to factors other than intrinsic toxicity of the material. To systematically assess potential health effects of engineered nanomaterials, inhalation exposure was carried out to investigate the distribution of gold in various organs of rats. Possible changes in gene expression of the lung / kidney were also examined.
Adult male Wistar rats were exposed to airborne nano-sized particles (NSPs) (2 x 10^6 #NSPs/cm3) for 6 hours per day for 5 or 15 days. Accumulated gold in tissue samples were quantified using ICP-MS.
After 5-day exposure, significant accumulation of gold was detected in the lung (100 ng Au/g tissue) and olfactory bulb (8 ng Au/g tissue). After 15-day exposure, significant accumulation of Au was detected in over 20 organ and tissues. Of the above tissues, the highest concentrations of gold were substantially observed in lung, esophagus, tongue, kidney, aorta, spleen, septum, heart and blood. In the lung, genes that showed the greatest downregulation (>10 folds) were related to muscle. The lung and kidney showed significant downregulation in more than two genes. Expression of the gene for thyroid hormone responsive protein in the kidney was also significantly decreased.
Inhaled nAu is able to translocate from the lung to other organs with time, and can cause significant genetic changes in exposed tissues. Findings of this work pave ways to study toxicity mechanisms and effects of function of inhaled nAu on targeted organs.

1399 USE OF LABELED SINGLE WALLED CARBON NANOTUBES TO STUDY ACUTE TRANSLOCATION FROM THE LUNGS.

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Single walled carbon nanotubes (SWCNT) have a small size and unique physical-chemical properties which suggest that novel particles may translocate to systemic organs after deposition in the lungs. In this study, gold particles (10nm) were used to label a highly dispersed preparation of SWCNT with a mean geometric diameter of 1.1 microns. The gold labeled SWCNT were delivered to the lungs by pharyngeal aspiration to C57BL/6 mice. Neutron activation analysis (NAA) of lung, blood and other organs was carried out at various times after aspiration to determine if the gold labeled SWCNT translocated out of the lungs. Six mice per group were studied at 1 hour, 1 day and 3 days after exposure to a single dose of 50 μg of gold labeled SWCNT. A separate group was given gold particles without SWCNT. A phosphate buffered saline (PBS) aspiration group served as the negative control. At sacrifice the lungs, heart, brain, liver, kidneys and a blood sample were taken for analysis of gold content by NAA. Gold content of the PBS aspiration group was negligible. In the gold labeled SWCNT group lung gold content at 1 day was 65 percent of that at 1 hour and not significantly different from the content at 3 days. The heart, brain, liver, kidney and blood samples from the gold labeled SWCNT groups contained less than 1 part in 200 of the total dose and were not statistically different from the PBS aspiration group at 1 hour, 1 day and 3 days. The results indicate that SWCNT do not readily translocate from the lungs.

1400 SHORT-TERM INHALATION TESTS WITH EIGHT NANO-MATERIALS IN RATS.

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Inhalation exposure is the route of highest concern for nano-particles and nano-objects. BASF has developed a standard short-term inhalation test to examine the toxicity of inhaled aerosols from nanomaterials. The generation of atmospheres from nanomaterials and the characterization of these atmospheres has been investigated: Aerosols from nanomaterials are complex mixtures comprising of a large fraction of agglomerated and aggregated particles and – to a minor extent – of actual ultrafine particles. To estimate the toxicity of the inhaled aerosols from nanomaterials an extensive set of parameters has been evaluated an most sensitive and predictive parameters have been identified (SOT 2006).

Applying the standard short-term inhalation test for nanomaterials, we tested eight nano-materials, TiO2, ZnO, CeO2, ZrO2, amorphous SiO2, coated amorphous SiO2, carbon black and multi-wall carbon nano tubes, in male Wistar rats. Rats were exposure to three concentrations for six hours on five consecutive days. At the end of the exposure, and after a recovery period of two to three weeks, animals were examined:

Organ burdens were estimated in seven tissues. Electron microscopy was carried out to characterize the particles deposited in the lung and to determine possible translocation of the materials. Moreover, the respiratory tract of the animals, as well as all organs and tissues with macroscopic changes were examined by histopathology. Proliferation and apoptosis was examined in the lung. The complete set of hematology and clinical chemical parameters in blood were examined. Additionally, an extensive set of cytokines and chemokines as well as standard clinical pathology parameters were analyzed in lung lavage fluid and blood.

The lung and body distribution, the lung effects and NOAEC differed among the eight nanomaterials indicating effects of the agglomeration state, the surface area and the surface chemistry on the fate and effect in the lung.

1401 ADSORBED PROTEINS INFLUENCE THE BIOLOGICAL ACTIVITY AND MOLECULAR TARGETING OF NANOMATERIALS.

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The possible combination of specific physicochemical properties operating at unique sites of action within cells and tissues has led to uncertainty surrounding nanomaterial toxic potential. We have investigated the importance of proteins adsorbed onto the surface of two distinct classes of nanomaterials (single walled carbon nanotubes, SWCNTS; 10 nm amorphous silica) in guiding nanomaterial uptake or toxicity in the RAW 264.7 macrophage-like model. Albumin was identified as the major fetal bovine or human serum/plasma protein adsorbed onto SWCNTs, while a distinct protein adsorption profile was associated with Nagase Analbuminemic Rat (NAR) plasma. Damaged or structurally altered albumin is cleared from systemic circulation by scavenger receptors. We observed that SWCNTs inhibited the induction of cyclooxygenase-2 (Cox-2) by lipopolysaccharide (LPS; 1 ng/ml, 6 hr) and this anti-inflammatory response was inhibited by fucoidan (scavenger receptor antagonist) which also reduced the uptake of fluorescent SWCNTs (Alexa647). Precoating SWCNTs with a non-ionic surfactant (Pluronic F127) inhibited albumin adsorption and anti-inflammatory properties. Albumin-coated SWCNTs reduced LPS-mediated Cox-2 induction under serum-free conditions. SWCNTs did not reduce binding of LPSAlexa488 to RAW 264.7 cells. The profile of proteins adsorbed onto amorphous silica particles (50 – 1000 nm) was qualitatively different, relative to SWCNTs, and precoating amorphous silica with Pluronic F127 dramatically reduced the adsorption of serum proteins and toxicity. Collectively, these observations suggest an important role for adsorbed proteins in modulating the uptake and toxicity of SWCNTs and nano-sized amorphous silica. Research supported by U.S. DOE funding (Contract DE-AC05-76RL01830).

1402 PHARMACOKINETIC MODELLING AND IMPLICATIONS OF PERIODICITY IN ARTERIAL EXTRACTION OF QUANTUM DOT NANOPARTICLES.

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We have recently shown that quantum dots (QD) have a unique periodicity in arterial to venous extraction into skin that has not been seen with drug infusion (Lee at al. NanoLetters 7:2865, 2007), suggesting a potentially important nanomaterial behavior that could affect systemic disposition. The purpose of the present series of studies was to further probe this phenomenon using mathematical models. This extraction was modeled using first order ordinary differential equations (ODEs) based on a compartmental model. The periodicity could only be modeled when it controlled release from the tissue to venous compartment, and not by release from the arterial to tissue compartment. There was no periodic orbit for an autonomous nonlinear compartmental model, suggesting that a non-autonomous model best fits the data. Also, we used a PBPK (physiologically based pharmacokinetic) model to simulate effects of this periodicity in arterial to venous extraction on systemic disposition. The PBPK models consist of five tissue blocks (skin, muscle, liver, kidney, lumped tissues). The tissues are assumed to be a homogeneous well-mixed space with respect to concentration of the drug. For the purpose of this simulation, periodicity was only associated with skin and muscle. Time-dependent tissue redistribution from tissue masses including skin into the liver and kidneys could be driven by such a periodicity in arterial extraction. These simulations defined the amplitude in periodicity required to be detected in a PBPK model and suggest experimental designs required to detect it. (Supported by US EPA STAR Program RD 8317150).

1403 COMPARATIVE BIODISTRIBUTION OF COATED AND UNCOATED NANO- AND MICRON-SIZED TITANIUM DIOXIDE FOLLOWING INTRADERMAL ADMINISTRATION IN MICE.

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The current inclusion of nanoscale titanium dioxide (TiO_2) and zinc oxide in personal care products, namely sunscreens and cosmetics, has aroused much debate regarding their safety. As an initial step for evaluation of the potential toxicity of nano-TiO_2, we have sought to determine the bioaccumulation of TiO_2 in internal organs following deliberate introduction into the skin (i.e. determination of sentinel organs). Hairless mice were intradermally injected (5 µL) with aqueous suspensions of nanoscale (25 nm primary particle size) uncoated anatase (nA)-TiO_2)(0.05-2 mg/mL), microscale rutile (mR)-TiO_2 (2 mg/mL) and nanoscale (14 x 79 nm rods) polystearate/alumina-coated (nR)-TiO_2 (2 mg/mL). Blood, skin at the site of injection, distal control skin, regional lymph nodes, and liver were collected at 6, 24 and 48 hours post injection and titanium (Ti) levels quantified by ICP-MS. A dose-dependent increase in Ti at the site of injection was observed with nA-TiO_2 with no change over 48 hours. Similarly, the Ti levels were unchanged between 6-48 hours at the site of injection with the nR- and mR-TiO2. Ti levels were elevated in the lymph nodes after injection with nR-TiO_2 (24, 48 hrs) and mR-TiO2 (6, 48 hrs) but not elevated following injection with the nA-TiO_2. Liver Ti levels were elevated 24 and 48 hrs following injection with 2 mg/mL of all three TiO_2's, and at 24 hours with 0.5-2 mg/mL nA-TiO_2, but not in a dose-dependent manner. Blood titanium levels did not correlate with tissue levels. These results suggest that the biodistribution of TiO_2 following intradermal injection depends upon the size and surface properties with lymph nodes and liver being potential sentinel organs following intradermal injection of TiO_2.

1404 BIOKINETICS OF AU NANOPARTICLES RELATIVE TO SIZE, SURFACE COATING AND PORTAL OF ENTRY.

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We hypothesized that potential adverse health effects of engineered nanoparticles (NPs) depend on the portal of entry, their surface coating, and subsequent distribution to different body compartments. At 24-hours post exposure, we examined the disposition and tissue retention of model Au NPs administered intratracheally (IT) or by intravenous injection (IV). Three different sizes (5, 50 and 200 nm) and 3 different coatings were used: citrate, serum albumin, and 5 and 20 kD polyethylene glycol (PEG). Depending on the portal of entry, we found major differences in the distribution of these particles relative to size and surface coating. The majority of the albumin-coated and citrated particles, for all sizes, were retained in the liver when delivered IV. In contrast, the liver was not a major organ of uptake for NPs that translocated systemically after being delivered IT. Most of the PEG-coated Au NPs entering the systemic circulation were retained there at 24 hours after either route of exposure, except for the PEG-coated Au 200 nm particles, which were retained in the liver. More of the 5 nm Au particles administered IT vs. IV were

found in the brain. The reverse was true for 5 nm PEG-coated Au particles. In conclusion, primary NP coating determines organ distribution when administered IV. However, we suggest that secondary coatings occur at the portal of entry when NPs are administered IT that seem to alter these distribution kinetics. Overall, the portal of entry, coating, and size are key factors that affect NP biokinetics.
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1405 NANOMATERIAL WITH A SPECIFIC SET OF PROPERTIES CAN ENTER THE CELL *VIA* MULTIPLE PATHWAYS.

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Submicron and nanoscale particles that enter the respiratory tract are likely to reach the alveoli, where alveolar type II epithelial cells that are distinguished by apical microvilli are found. These cells play critical roles in the function of the alveoli, and participate in the immune response to certain particles by releasing chemokines. However, the cellular interactions and internalization pathways of the particles, which drive the cellular response, are still unclear. To investigate the size and surface dependent interactions of submicron and nanoscale amorphous silica particles with alveolar type II epithelial cells we used a non-tumorigenic cell line (C10), which expresses villin and develops microvilli in our cultures. Previously, we reported a new retrograde recruitment pathway of the particles along microvilli-like structures toward the cell body of these cells. The retrograde motion, which is strictly dependent on the positive surface charge of the particle, is powered by the retrograde actin flow and reveals the coupling of the particle with the actin filaments across the plasma membrane. Using time-lapse fluorescence imaging we observed that certain heparan sulfate proteoglycans can serve as mediators in this coupling. Using fluorescent transferrin we show that the positively charged (500nm -100nm) particles can enter the cell via clathrin-dependent endocytosis, which also requires intact actin dynamics. However, the internalization of the particles via interactions and pathways that are independent of actin dynamics or the integrity of the filaments is also demonstrated using cytochalasin D. Our observations demonstrate that while surface properties and size dictate the cellular interactions and internalization pathways of the particles, multiple internalization pathways are available for particles with a specific set of properties.
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1406 MACROPHAGE UPTAKE OF 26 NM AND 250 NM PSL IS MEDIATED BY SEPARATE MECHANISMS.

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The rates, that 26 nm and 250 nm diameter polystyrene latex (PSL) beads are accumulated by macrophages, should reflect differences in molecular and cellular function. We used a confocal microscope to measure the accumulation-rates of nanoparticles by F344-rat-alveolar macrophages (approximately 25,000 cells adhered to a 0.7 cm^2 well). Over the cells were layered 0.1 ml of media, and 0.1 ml of media-with-beads. Fresh cells were introduced for each exposure scenario. The number concentrations were as follows: 26 nm beads at 1.0E+12, 26 nm beads at 1.0E+11, and 250 nm beads at 1.4E+09 beads per ml (a number ratio of 714 to 71 to 1). For each scenario, average accumulation-rates were estimated over 23 minutes. At 3 minutes after introduction of beads, macrophages (in the respective scenarios) contained 144871, 12909, and 22 beads/cell and had accumulation-rates of 30947, 3159, and 7.9 beads per min. At 5 minutes macrophages contained 198155, 19617, and 40 beads/cell and had accumulation-rates of 24799, 3392, and 8/min. At 15 minutes macrophages contained 385543, 52644, and 147 beads and had accumulation-rates of 14204, 2718, and 12.8 /min. Comparisons were made against macrophages exposed to the lower concentration of 26 nm beads. For the high concentration of 26 nm diameter beads, at 3 minutes, the accumulation-rate was as expected; however, the accumulation-rate for 250 nm diameter beads was low (18% of expected). At 15 minutes, macrophages, exposed to the higher concentration of 26 nm dimaeter beads, had lower than expected accumulation-rates; by comparison, the accumulation-rate for 250 nm diameter beads, although still low (34% of expected), had almost doubled. These measurements imply that after a macrophage accumulates over 200,000 of the 26 nm diameter beads, the accumulation-rate for these beads begins to decrease, possibly due to colligative effects. On the other hand, for 250 nm diameter beads, the observed increase in accumulation-rate is most likely due to activation of different endocytotic processes (see Radcliff et al, Toxicologist 2008).

1407 CIGARETTE SMOKE (CS) CAUSES FORMATION OF REGIONS OF ALTERED DNA METHYLATION (RAMS) IN SENCAR MOUSE LUNG.

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DNA methylation is an epigenetic modification regulating gene expression and aberrant methylation patterns contribute to tumorigenesis and other toxicities. Female SENCAR mice were exposed to air only, or treated once with 10 mg/kg/ip 7,12-dimethylbenz-α-anthracene (DMBA), or with DMBA plus 0.16, 0.32 or 0.48 mg wet total particulate matter/L (WTPM/L) CS in air, 3 hr/day, 5 days/wk for 4 or 8 wk. RAMs were identified in lung DNA using methylation-sensitive restriction digestion, arbitrarily primed PCR and capillary electrophoretic separation of PCR products. RAMs which carry forward in time might have special toxicological significance. We hypothesized that more "carry forward" RAMs would be seen in the DMBA+CS group as compared to DMBA alone. RAMs that occurred in response to CS at 4 and 8 wk, in general, exhibited dose- and time-response relationships. Only 4% of the RAMs that formed due to DMBA alone at 4 wk were also observed at 8 wk ("carry forward" RAMs). A much higher percent of carry forward RAMs from 4 to 8 wk were discerned as a result of DMBA+CS: 0.16 mg, 26%; 0.32 mg, 37%; and 0.48 mg, 25%. Multiple RAMs seen in the 2 lower CS dose groups also formed in the high dose group at both 4 and 8 wk. Of the 143 RAMs that occurred due to 4-wk treatment with 0.48 mg CS, 68 occurred in 1 or both of the 2 lower dose groups while 75 unique RAMs were also detected in the 0.48 mg group. Similarly, of the 174 RAMs that occurred due to 8-wk treatment with 0.48 mg CS, 132 occurred in 1 or both of the 2 lower dose groups while 42 unique RAMs were also detected in the 0.48 mg group. Most of these alterations, at 4 and 8 wk, were hypomethylations. Histopathological changes in response to CS were mild, at most, and revealed dose- and time-response relationships. These data are consistent with the hypothesis that CS can promote progressive alterations of DNA methylation and this might be an early biomarker of CS-induced lung toxicity.

1408 VARIABLE HISTONE MODIFICATIONS AT THE A^{VY} METASTABLE EPIALLELE.

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The ability of environmental factors to shape health and disease involves epigenetic mechanisms that mediate gene-environment interactions. Metastable epialleles are alleles that are variably expressed in genetically identical individuals due to epigenetic modifications established during early development. The extent of DNA methylation within metastable epialleles is stochastic due to probabilistic reprogramming of epigenetic marks during embryogenesis. Stochastic methylation patterns are also influenced by maternal nutrition and environment during early development. Little is known, however, about the role of histone modifications in metastable epiallele expression and phenotypic variation. We utilized chromatin immunoprecipitation for key histone modifications on liver tissue followed by qPCR to characterize variable histone marks in the 5' long terminal repeat (LTR) of the murine viable yellow agouti (A^{vy}) metastable epiallele. This region contains 9 CpG sites, which are variably methylated in isogenic A^{vy}/a offspring of different coat colors. We specifically investigated sites 4 to 9, a region postulated to contain the highest probability of modified histone binding sites. Herein, we report that yellow mice, which are hypomethylated at the A^{vy} LTR, display enrichment of H3 di-acetylation (p-value<0.05). Pseudoagouti mice, in which A^{vy} hypermethylation is thought to silence ectopic expression, exhibit enrichment of H4K20 tri-methylation (p-value<0.05). No differences among coat color classes were observed for H3K4 tri-methylation (p-value>0.4), a modification often enriched in the promoter region of active genes. These results show for the first time the presence of variable histone modifications at a metastable epiallele, indicating that DNA methylation acts in concert with histone modifications to affect inter-individual variation in expression. Therefore, the potential for environmental factors to influence variable histone modifications, in addition to DNA methylation, should be addressed in environmental epigenomic studies.

1409 MEASUREMENT OF CIGARETTE SMOKE CONDENSATE-INDUCED STRUCTURAL AND FUNCTIONAL ALTERATIONS IN BRONCHIAL CELLS USING QUANTITATIVE HIGH CONTENT SCREENING.

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We hypothesized that cigarette smoke condensate (CSC) activates protein kinase C-alpha (PKC-α)concurrent with disruption of the actin cytoskeleton and altered cell function in human BEAS-2B immortalized, non-cancerous bronchial epithelial

cells. F-actin distribution and amount, and PKC-α activation were assessed in control and CSC-treated BEAS-2B cells using high content screening kits and a Cellomics ArrayScan® automated imaging system. Control BEAS-2B cells displayed F-actin organized as stress fibers near the cell membrane and occasionally throughout the cells. When cells were treated with CSC, peripheral stress fibers were reduced and there was a shift in F-actin distribution whereby disrupted actin aggregates occurred at the cell periphery along with perinuclear actin, and actin filaments scattered throughout the cells. Treated cells also possessed numerous cell surface extensions including elongated filopodia. CSC exposure for 24 hrs followed by cytotoxicity evaluation using the neutral red assay showed that the IC50 of CSC in BEAS-2B cells was 19 μg/ml. PKC-α was activated when BEAS-2B cells were treated with doses of CSC ranging from 10-50 μg/ml. Activation of PKC-α occurred concurrent with increased cell adhesion and decreased cell spreading. Detachment assays showed that cells treated with 20 and 30 μg/ml CSC for 3 hrs displayed significantly ($p<0.05$) greater adhesion to the substrate than vehicle control-treated cells. In fact, treatment of cells with doses of 20 and 30 μg/ml CSC induced twice as many cells to attach to the substrate compared to vehicle controls. PKC-α inhibition in BEAS-2B cells induced by treatment with 7 nM of the indolocarbazole Gö6976 restored F-actin patterns, cell detachment and cell spreading to levels similar to control cells. Alterations in signaling pathways induced by CSC concurrent with alterations in bronchial cell structure and function may impair the ability of these cells to serve their normal protective role.

1410 IDENTIFICATION OF EARLY MOLECULAR EVENTS UPON PEROXISOME PROLIFERATOR EXPOSURE IN THE RODENT LIVER.

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Peroxisome proliferators (PP) are a large class of structurally diverse chemicals that mediate their effects in the liver mainly through the PP-activated receptor α (PPARα). Development of PP-induced hepatocarcinogenesis in mouse liver is known to be dependent on PPARα but molecular events which precede the alteration in cell proliferation and apoptosis are not well defined. Microarray data sets collected from the livers of mice and rats treated with three different PP for 12 to 24 hours were used in Gene Set Enrichment Analysis (Broad Institute, MIT, Boston MA) to elucidate the mechanisms of early responses. We identified a number of common enriched gene sets from GSEA analysis. These included those that were up-regulated (cell cycle, DNA replication, Myc, fetal liver vs adult liver, statin pathway) and down-regulated (pyruvate metabolism, TNFalpha, Brca1, etc.). These common gene sets will be examined using a number of pathway tools (e.g., Ingenuity Pathway Analysis) to help to understand the relationships between the regulation of these sets of genes and the increases in cell proliferation and decreases in apoptosis that have been associated with PP-induced carcinogenesis. This abstract does not necessarily reflect EPA policy.

1411 THE ROLE OF TUMOR PROMOTER-INDUCED PKC ACTIVATION IN THE REGULATION OF HUMAN EQUILIBRATIVE NUCLEOSIDE TRANSPORTER 1 EXPRESSION AND ACTIVITY.

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Equilibrative nucleoside transporters play an important role in cell physiology by regulating extracellular nucleoside concentrations. These transporters are also necessary for the uptake of nucleoside analogs, which are commonly used as chemotherapeutic and antiviral agents. Despite their importance, little is known about the regulation of human ENT1 (hENT1) expression and activity. Current studies have suggested that estrogenic compounds, such as tetra-bromo bisphenol A (TBBPA), can activate Protein Kinase C (PKC), which has been implicated in the regulation of hENT1. This compound is a suspected tumor promoter, and interestingly, we found that it increases hENT1 activity in human breast cancer cells. The goal of this project was to further investigate the role of tumor promoter-induced activation of PKC and downstream signaling pathways in the regulation of hENT1 expression and activity. MDA MB 231 breast cancer cells were treated with TPA (a tumor promoter and known activator of PKC) as well as with TBBPA. ENT1 activity was measured, using radio-labeled uridine uptake. In addition, expression was analyzed using promoter-reporter assays, and quantitative real-time PCR. Results from these experiments indicate that both hENT1 expression and activity are significantly increased after treatment with TPA, and that these events are ERK dependent. Taken together these data suggest that phorbol esters (TPA), through activation of PKC and ERK signaling pathways, modulate hENT1 activity and expression.

1412 CONSTITUTIVELY ACTIVE B-RAF DOES NOT REGULATE CYCLIN D1 IN 2, 3, 5-TRIS-(GLUTATHION-S-YL) HYDROQUINONE TRANSFORMED, TUBEROUS SCLEROSIS-2 NULL CELLS.

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2,3,5-Tris-(glutathion-S-yl) hydroquinone (TGHQ), a hepatic metabolite of hydroquinone, is a renal carcinogen in Eker rats. Treatment of Eker rats with TGHQ results in loss of the wild-type allele of the tumor suppressor tuberous sclerosis-2 (TSC-2) in renal preneoplastic lesions and tumors. The development of these renal tumors is thought to be a consequence of disruption of the TSC-2/TSC-1 complex, which is a known inhibitor of TOR signaling. The tumor suppressor activity of TSC-2/TSC-1 may also negatively regulate the B-RAF/ERK/MAPK cascade. TGHQ transformed primary renal epithelial cells from Tsc-2 EK/+ rats (QTRRE cells) are null of tuberin due to loss of heterozygosity at the Tsc-2 allele, and exhibit high ERK and B-Raf kinase activity. Concomitant with high B-Raf kinase activity, QTRRE cells express constitutively phosphorylated B-Raf at amino acid residues S345 and S483 (LC-MS/MS). Coimmunoprecipitation revealed that within the cytosol of QTRRE cells, the active B-Raf forms a trimeric protein complex with Raf-1 and 14-3-3 isoforms. Use of the broad spectrum kinase inhibitor BAY 43-9006, which inhibits in order of potency: C-Raf, VEGFR3, B-Raf, PDGFR, Flt3, c-Kit, and VEGFR2; revealed decreased activity of ERK1/2 and decreased expression of cyclin D1. In contrast, although treatment with the MEK1/2 inhibitor PD 98059 resulted in a decrease in ERK1/2 phosphorylation, cyclin D1 protein expression remained unchanged. Following siRNA knockdown of B-Raf, Western blot analysis revealed a decrease in B-Raf protein expression but no significant decrease in MEK1/2 phosphorylation or cyclin D1 protein expression. Therefore, the data suggest that the 14-3-3/B-Raf/Raf-1 protein complex does not drive the increased expression of cyclin D1 during renal proximal tubule tumor formation. Further mechanistic studies of the 14-3-3/B-Raf/Raf-1 protein trimer may elucidate the mechanism leading to renal tumor formation.(GM39338, GM070890, ES006694, ES06694)

1413 PROAPOPTOTIC INTERACTIONS OF NON-PLANAR PCBs IN RAT LIVER EPITHELIAL CELLS: ROLE OF MITOGEN-ACTIVATED PROTEIN KINASES.

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Non-planar polychlorinated biphenyls (N-PCBs) are the predominant congeners of environmental PCB mixtures that exert their toxic effects though Ah-receptor independent mechanisms. Ongoing studies in our laboratory have identified that the diortho-chlorinated 2,2',4,4'-tetrachlorobiphenyl (TCB) activates the extracellular-regulated kinase (ERK). To understand the biological impact of ERK activation we have examined the expression of genes that are mediated through activated ERK and genes that are expressed independent of ERK in WB F-344 (WB) cell line derived from rat liver. Microarray analysis of one hundred genes revealed that ERK-dependent activation of the immediate early genes c-fos, c-jun and EGR-1 as well as the proinflammatory gene cox-2 in WB cultures treated with 20.0 μM TCB for one hour. Expression of these was inhibited by treatment of the cells with MAPKK inhibitor U-0126. TCB treatment also enhanced two cell cycle related genes cyclin D1 and cdkn1b at 2 and 4 hrs post treatment. Cell cycle analysis of TCB treated cells did not induce progression to S phase where significant induction of S phase cells was observed in cells treated with the mitogen TGF-α. Microarray analysis of the expression of genes related to apoptosis in cells treated with TCB for 4 hrs revealed that the chemical induced the expression of both pro- and anti-apoptotic genes of Bcl-2 and caspase, p53 ATM, and TNF receptor families. Taken together these data suggest that the non-planar TCB activates ERK-MAPKs leading to the induction of some inflammation associated genes encoding transcription factors as well as genes that are either proapoptotic or anti-apoptotic and that the epigenetic effects of N-PCBs is mediated by altered expression of gene. (supported by NIEHS grants ES-04911 and Philip Morris External Research Program grant).

1414 17α-ETHINYLESTRADIOL ESPOSURE HINDERS HEPATIC NUCLEOTIDE EXCISION REPAIR IN ZEBRAFISH.

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Estrogen is a known human carcinogen and is implicated in the etiology of human hepatocellular carcinoma. However, current research does not adequately address the role of estrogens as regulators of hepatic nucleotide excision repair (NER). NER is exceedingly pertinent in HCC etiology because this coordinated process repairs DNA lesions such as those caused by aflatoxin B1, the foremost epidemiological cause of hepatocellular carcinoma (HCC) worldwide. Zebrafish exposed to the

strong estrogen agonist 17α-ethinylestradiol displayed decreased mRNA expression of multiple nucleotide excision repair (NER) genes in vitro and in vivo. Additionally, liver derived zebrafish cell lines treated with estrogens were hindered in their ability to repair bulky DNA adducts. Both of these phenomenon were significantly more prevalent in male zebrafish which parallels the incidence of HCC in the human population. These data suggest that estrogen can act as a co-carcinogen with bulky adduct forming mutagens by hindering a cells ability to deal with DNA damage.

1415 INTERACTION BETWEEN THE ARYL HYDROCARBON RECEPTOR (AHR) AND THE RAS/RAF SIGNALING PATHWAYS IS REQUIRED FOR AHR- INDUCED EXPRESSION OF MATRIX METALLOPROTEINASES

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Malignant melanoma has a high incidence of mortality due to its resistance to chemotherapy and tendency towards metastasis and in the past 60 years has seen an increase in industrialized nations. Melanoma progression involves a transition to malignancy involving matrix metalloproteinase (MMP) expression. MMPs are a family of zinc- and calcium-dependent proteinases classified by extracellular matrix substrate specificity and are essential in cancer progression. The aryl hydrocarbon receptor (AhR) originally identified as the receptor for the polycyclic aromatic hydrocarbon (PAH) family of environmental contaminants, binds endogenous and exogenous compounds, including flavonoids, UV photoproducts of tryptophan, as well as some synthetic retinoids. We have demonstrated that activation of the AhR pathway results in increased expression and activity of several MMPs. The goal of this study is to determine whether AhR-induced MMP expression requires additional cell signaling events. We show that TCDD-induced MMP expression requires the AhR signaling pathway through use of chemical inhibitor as well as siRNA to AhR. We also show that Ras activation is critical for AhR-induced MMP expression. Interestingly, 60% of all melanomas contain an activating mutation, V599EBRAF, in the Ras/Raf pathway. We demonstrate that AhR-induced MMP-1, -2 & -9 expression also depends on Ras/Raf signaling. Crosstalk between Ras/Raf signaling and the AhR pathway is illustrated by a Erk phosphorylation reduction by AhR inhibition. Inhibition of Ras/Raf signaling using the MEK inhibitor, U0126, results in down-regulation of AhR while AhRs competitive inhibitor, aryl hydrocarbon receptor repressor (AhRR) is up-regulated. These data demonstrate that TCDD-induced expression of MMPs in A2058 cells require Ras/Raf signaling as well as the AhR pathway and that the two pathways may directly affect one another.

1416 MICRORNAOME CHANGES AND BREAST CANCER DRUG RESISTANCE.

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Many human cancers, including breast cancer, fail respond effectively to chemotherapy, and cancers that initially respond often acquire drug resistance. We have recently shown that epigenetic changes play crucial role in resistance of breast cancer cells to doxorubicin and cisplatinum. Another epigenetic mechanism of gene expression control via the function of small RNAs, particularly – microRNAs (miRNAs) remained unexplored. MiRNAs are evolutionally conserved small single-stranded non-protein-coding RNA molecules presently recognized as major negative gene regulators. They play crucial role in regulating cellular differentiation, proliferation, and apoptosis.

We hypothesized that microRNAome changes are important in the resistance of cancer cells to chemotherapeutic drugs.

MiRNA microarrays were used to analyze the miRNA expression patterns in the human breast adenocarcinoma MCF-7 cell line and its drug-resistant to doxorubicin MCF-7/DOX and to cis-Dichlorodiammine platinum (II) MCF-7/cisDDP variants. Microarray data were confirmed using qRT-PCR. The cluster analysis revealed that MCF-7/DOX and MCF-7/cisDDP cells are characterized by significant change in the miRNAome profiles. Importantly, in addition to the distinctive microRNAome signatures of MCF-7/DOX and MCF-7/cisDDP cells, we identified 35 down-regulated and 25 up-regulated miRNAs with similar expression patterns in both MCF-7/DOX and MCF-7/cisDDP resistant cells at a level of $p < 0.01$. Cluster of down-regulated miRNAs in drug-resistant cells consisted of, for instance, let 7a, let 7f, let 7d, mir-34a, mir-15b and mir-149 controlling expression of MRP1, MDR1, BRCA1, and BRCA2; mir-27b regulating CYP3A. In contrast, number of microRNAs, such as mir-214, mir -28, let-7i, cluster mir-17-92 regulating apoptosis-related genes, was up-regulated in resistant cells.

Importantly, using the reporter gene-3'UTR fusions, we have confirmed the microRNA species that target MDR1 and MRP1, thereby regulating their expression. Our results indicate the importance of microRNAome deregulation in development of cancer drug resistance.

1417 OLD NSAID BUT NEW ANTICANCER AGENT: TOLFENAMIC ACID INHIBITS PANCREATIC CANCER GROWTH AND SENSETIZES TUMOR TO RADIOTHERAPY THROUGH DOWNREGULATION OF SP PROTEINS.

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Sp1, Sp3 and Sp4 proteins play a critical role in the growth and metastasis of pancreatic cancer cells. Initial studies screened a panel of nonsteroidal anti-inflammatory drugs (NSAIDs) and cyclooxygenase 2 (COX-2) and COX-1 inhibitors for their effects on Sp protein expression. Only tolfenamic acid and its derivatives degraded all three Sp proteins. The most active compound was tolfenamic acid which also inhibited growth of Panc-1, L3.6pl and Panc-28 pancreatic cancer cells. Tolfenamic acid induced proteasome-dependent degradation of Sp1, Sp2 and Sp3. Tolfenamic acid also inhibited Vascular Endothelial Growth Factor (VEGF) and the antiapoptotic survivin mRNA/protein expression in Panc-1 and L3.6pl cells, and this was linked to decreased binding to and activation of proximal GC-rich sites in the VEGF and survivn promoter. Orthotopic model for pancreatic tumor growth has been used to investigate tolfenamic acid-induced antiangiogenic and proapoptotic responses. Treatment with 12.5 and 25 mg/kg tolfenamic acid decreased tumor growth and weight compared to solvent (control) and also decreased liver metastasis. In addition, tolfenamic acid increase sensitivity of pancreatic cancer cells and tumor to radiation therapy and this was mediated by blocking radiation-induced survivn protein overexpression. Phase I clinical trial with tolfenamic acid is on going at M. D. Anderson Cancer Center Orlando on patients with pancreatic, colon and prostate cancers. Thus, tolfenamic acid acts as a novel anticancer agent and radiation sensitizer through targeted degradation of Sp proteins that are highly overexpressed in tumor.

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1418 BORIC ACID AS A NOVEL ENDOPLASMIC RETICULUM MODULATOR AND CALCIUM RELEASE ANTAGONIST IN DU-145 PROSTATE CANCER CELLS.

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Boron is a naturally occurring, ubiquitous, trace element found in physiological systems as boric acid. It is essential for plant growth and can be found in fruits, nuts, legumes, vegetables, and wine. Boric acid has been shown to decrease the risk for prostate cancer in epidemiological studies, exhibiting a dose response between dietary boron intake and decreased risk of prostate cancer. Boric acid supplementation has also been shown to inhibit the growth of prostate tumors in mice and proliferation of DU-145 and LNCAP prostate cancer cell lines over an 8 day period. The purpose of this study was to determine boric acid's site and mechanism of action in DU-145 prostate cancer cells. We began with a broad proteomics approach and found that low doses of boric acid cause endoplasmic reticulum (ER) stress in DU-145 prostate cancer cells, inducing the unfolded protein response (UPR). Through 2 dimensional (2D) proteomics and western blot analysis, we have shown that hallmarks of the UPR, Bip, calreticulin, and phosphorylation of eiF2α, are up regulated in response to boric acid treatment over 24 hours. In addition, the ER becomes swollen and vacuolated in treated cells. Boric acid also rescues cell death and calcium depletion in the ER in response to thapsigargin, a SERCA blocker, without effects on SERCA function. We also found that boric acid acts as a calcium release antagonist in response to ryanodine receptor agonists, caffeine and 4-chloro-m-cresol. We demonstrate that the ER ryanodine receptor is a primary site of action for boric acid and alteration of calcium homeostasis is a plausible mechanism for boric acid induced ER stress and UPR induced inhibition of prostate cancer cell growth. Funded by TSRTP and US Department of Defense.

1419 EFFECT OF SODIUM ARSENITE ON METALLOTHIONEIN 1/2 AND P53 PROTEIN LEVELS AND ITS POTENTIAL RELATIONSHIP WITH CELL CYCLE ALTERATIONS IN MCF-7 CELLS.

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Arsenic exposure has been associated with an increased risk of skin, urinary bladder, lung and liver cancer. However, little is known about the mechanisms of As carcinogenesis. p53 protects cell integrity by preventing cell cycle progression when the cell has undergone DNA damage. The presence of metallothionein (MT) has also been related with proliferation and apoptosis prevention. It is known that As induces p53 and the expression of MT isoforms in several human cell lines. Recent studies have shown that MT 1/2 interact with p53 and have suggested that this interaction could regulate the binding of p53 to DNA. The aim of this study is to evaluate the effects of sodium arsenite (0-10 μM) on MT 1/2 and p53 protein levels and their possible relationship with cell cycle alterations in the human breast cell line MCF-7. Cells exposed to low As doses presented an increased growth rate, as measured by trypan blue exclusion test. An analysis by FACS showed an increased recruitment of cells in the S phase of the cell cycle. In addition, treated cells showed concentration dependent increases in p53, MT 1/2 and PCNA protein levels, as measured by Western blot. These findings suggest that MT induced by arsenic prevents p53 to stop cell cycle progression, therefore resulting in cell proliferation.

1420 AHR PROTEIN INTERACTIONS: BUILDING A COMPREHENSIVE PROTEIN INTERACTION NETWORK.

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Dioxin and dioxin-like compounds are prevalent environmental contaminates produced by both natural and man-made activities. Adverse biological responses to these compounds are mediated through the Aryl-Hydrocarbon Receptor (AHR), a ligand activated transcription factor and a member of the PAS Super-family. The underlying mechanisms of toxicity induced by ligand activated AHR is still poorly understood. To explore the potential influences of protein-protein interactions in AHR-mediated toxicity, a Tandem Affinity Purification (TAP-tagging) and mass spectrometry strategy was utilized to isolate and identify proteins that co-purify with the AHR. The known protein interactions between the AHR, Hsp90, and ARA9 and the ligand-dependent interaction with ARNT have been identified, demonstrating the validity of the approach. Several unique protein interactions have also been identified using this approach and the functional significance of these interactions are under investigation. In parallel with the experimental activities, custom bioinformatics software is being developed to catalog, analyze, and visualize the protein interaction data generated by the study. The Protein Interaction Network Analysis Tools and Archive (PINATA) software is a client-server interface written in Java. PINATA tracks all experimental information results associated with a TAP-tag experiment, imports and labels gel images, and parses mass spectroscopy reports. A query interface is provided to compare protein interactions that are shared between samples. Ultimately, PINATA will create a dynamic network that reflects the changes in AHR protein interactions with treatment and time.

1421 IDENTIFYING THE MECHANISM OF AHR-MEDIATED OFF-TARGET EFFECTS INDUCED BY A-277249 USING CHIP-ON-CHIP TECHNOLOGY.

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A-277249 is a thienopyridine compound designed to downregulate the expression of adhesion molecules such as ICAM and VCAM, and could thus have potential therapeutic potential for a variety of inflammatory disorders. This compound was shown to cause adverse liver effects in Sprague-Dawley rats, including hypertrophy and liver enzyme elevations. Previous microarray analysis suggests that A-277249-induced hepatotoxicity is mediated by the activation of the aryl hydrocarbon receptor (AhR) pathway in rats. To further characterize the molecular mechanism of AhR activation, quantitative real-time PCR was used to evaluate the levels of CYP1A1 induction in hepatocytes from rat and human origin. A-277249 significantly up-regulated CYP1A1 in primary rat hepatocytes following 24 hours treatment. The effect is similar to that induced by the prototypical AhR ligand, 3-methylcholanthrene (3-MC). Interestingly, as opposed to rat, the magnitude of CYP1A1 induction by A-277249 in HepG2 and primary human hepatocytes was only less than 10% of that induced by 3-MC. Additional molecular techniques, such as chromatin immunoprecipitation and ChIP-on-chip, were employed to compare the profiles of AhR-mediated downstream genes by A-277249 and 3-MC. These results will provide insights into the mechanisms and species differences of AhR activation.

1422 TEMPORAL AND DOSE-DEPENDENT AHR-MEDIATED GENE EXPRESSION ANALYSIS IN HUMAN LIVER ADULT STEM CELLS.

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Time course and dose-response studies with the human liver cell line, HL1-1, which possesses stem cell characteristics were conducted to assess gene expression responses elicited by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). For the dose-response study, cells were treated for 12 hrs with 0.001, 0.01, 0.1, 1, 10 or 100 nM TCDD or DMSO vehicle control. The time course study was conducted by treating cells with 10 nM TCDD or vehicle for 1, 2, 4, 8, 12, 24 or 48 hrs. TCDD-elicited changes in gene expression were monitored using human cDNA microarrays consisting of 7,844 genes. Empirical Bayes analysis identified 251 genes that were differentially expressed at one or more time points. Most of those genes also exhibited dose-dependent responses. Dose- and time- dependent induction of CYP1B1, ALDH1A3 and SLC7A5 gene expressions were confirmed by qRT-PCR. Comparisons of the HL1-1 temporal responses with human HepG2 and mouse Hepa1c1c7 hepatoma cell lines identified 75 genes and 18 orthologs common to HL1-1 cells, respectively. Further comparison of temporal gene expression in HL1-1 with hepatic tissue from immature ovariectomized C57BL/6 mice treated with 30 μg/kg TCDD identified 32 commonly regulated orthologous genes, primarily associated with signal transduction and transcriptional regulation. Metabolism and transport related genes were also commonly regulated between all comparisons. This comparative model analysis further elucidates species-specific toxicity and susceptibility mediated by AhR-mediated gene expression, thus supporting high throughput screening assay development and more appropriate risk assessment while decreasing the uncertainties associated with extrapolation between species. Supported by NIGMS grant R21 GM075838.

1423 CHARACTERIZATION OF GENE EXPRESSION RESPONSES ELICITED BY TOXIC AND NON-TOXIC AHR LIGANDS IN MURINE HEPA1C1C7 CELLS.

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In order to characterize and distinguish the responses between toxic and non-toxic aryl hydrocarbon receptor (AhR)-mediated ligands, gene expression effects elicited by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 3,3',4,4',5-pentachlorobiphenyl (PCB126) were compared to indolo[3,2-b]carbazole (ICZ) and β-naphthoflavone (βNF) in murine Hepa1c1c7 hepatoma cells. Dose-response studies at 12 hrs confirmed lower EC50 gene expression values for TCDD and PCB126 when compared to ICZ and βNF. For example, Cyp1a1 EC50 values were 21 pM, 231 pM, 27.1 nM and 1.2 μM for TCDD, PCB126, ICZ and βNF respectively. Temporal gene expression profiling using cDNA microarrays was also performed with 10 nM TCDD, 1 μM ICZ, 10 μM βNF or vehicle (DMSO) for 1, 2, 4, 8, 12, 24 or 48 hrs. Ranking and prioritizing the 200 most responsive genes for each treatment resulted in only 36 genes regulated by all three compounds. In addition, 116, 91 and 103 compound-specific gene responses to TCDD, ICZ and βNF, respectively, were identified. Functional annotation of the gene responses using Gene Ontology revealed that the non-toxic AhR ligands, βNF and ICZ, resulted in enrichment of genes involved in protein and amino acid metabolism, whereas TCDD-specific responses resulted in the enrichment of genes related to DNA metabolism, replication and repair. These compound-specific responses and their related functional activity coupled with the differences in potency for common differentially expressed genes may provide key biomarkers in identifying responses specific to toxic AhR ligands. Supported by NIH T32 ES07255.

1424 CONSERVED AND DIVERGENT GENE EXPRESSION PROFILES DURING ETHYNYLESTRADIOL, TAMOXIFEN AND O, P'-DDT INDUCED UTEROTROPHY IN THE RAT UTERUS.

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Changes in uterine physiology, cell morphology and gene expression were evaluated in a comprehensive time course study after oral administration of 100 μg/kg bw ethynylestradiol (EE), 100 μg/kg tamoxifen (TAM) or 300 mg/kg o,p'-DDT (DDT) to immature, ovariectomized Sprague-Dawley rats. Animals were dosed once or once daily for 3 consecutive days and uteri were harvested 2, 4, 8, 12, 18, 24, or 72 hrs after treatment. EE, TAM, and DDT elicited uterotrophic EC50s of 16.2 μg/kg, 16.5 μg/kg and >64.6 mg/kg, respectively, in the dose response studies. TAM and DDT only induced a 3.3-fold increase in wet weight with a marked decrease in water imbibition compared to the 7-fold induction with EE. DDT treated histological samples were characterized by marked apoptosis in the luminal epithelia and stroma at 72 hr compared to EE while TAM treatment exhibited significantly higher induction of luminal epithelial cell height at 72hr. Temporal gene expression profiles were measured using custom rat cDNA microarrays consisting of 8,567 features representing 4,342 genes and identified conserved and divergently regulated genes. Of the 1984 genes commonly regulated by all three compounds, 1574 demonstrated highly correlated temporal profiles between all three datasets indicating a large degree of similarity in the expression profiles. However 10 and 183 genes exhibiting expression profiles unique from EE for TAM and DDT, respectively, suggest additional non-estrogen receptor mediated pathways being regulated by DDT but not TAM. Funded by US EPA RD 83184701.

1425 STEROIDOGENIC ACUTE REGULATORY PROTEIN AS A TARGET FOR ORGANOCHLORINE PESTICIDES IN LARGEMOUTH BASS (*MICROPTERUS SALMOIDES*).

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Steroidogenic acute regulatory protein (StAR), the protein involved in the rate-limiting step in steroidogenesis, transports cholesterol across the mitochondrial membrane. The organochlorine pesticides (OCPs) methoxychlor (MXC), dieldrin (DIEL), and p,p'-DDE (DDE) have been implicated in the disruption of steroid hormone production in fish in both field and laboratory studies, however the mechanisms of disruption are unknown. To determine if StAR is disrupted by exposure to OCPs, real-time quantitative PCR (qPCR) was used to quantify StAR mRNA expression in minced testis cultures from recrudescent largemouth bass (LMB). Cultures were exposed for 2 hours to 100 μM doses of MXC, DIEL, or DDE, and subsequently treated for 20 hours with 10 U/mL hCG, a potent activator of gonadotropin signaling and StAR expression. hCG stimulated StAR expression >2-fold under normal conditions. MXC exposure diminished the stimulatory response of StAR to hCG, whereas pretreatment with DIEL and DDE stimulated StAR expression under basal and hCG-treated conditions. Testosterone (T) levels in the culture medium was analyzed and exposure to MXC, DIEL, and DDE reduced hCG stimulated T synthesis by 28, 32, and 54%, respectively. None of the compounds significantly altered basal synthesis. To identify potential signal transduction pathways involved in OCP-mediated disruption of StAR expression, 5.6kb of the StAR promoter was sequenced and analyzed for putative transcription factor binding sites. *In silico* analysis revealed numerous sites, including 1 estrogen receptor (ER) and 4 androgen receptor (AR) sites. ER/AR mRNA expression was quantified by qPCR, although no clear correlation could be established between ER/AR and StAR expression and OCP exposure. These data suggest that StAR is a potential target for OCPs and that disruption of T synthesis in male LMB is mediated by signal transduction pathways other than classic steroid hormone receptor-mediated pathways.

1426 STRESS ASSAY ENSEMBLE RESPONSE TO ENVIRONMENTAL CHEMICALS IN DIFFERENT HUMAN CELL TYPES.

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The large number of environmental compounds that currently need characterization and prioritization for further toxicological study is a serious regulatory challenge facing the EPA. In addition to these agents comprising of pesticides, inerts, and high-production volume chemicals, new chemicals and novel materials of unknown toxicities such as nanoparticles are being introduced into the market each year. To meet this challenge, we are developing cell-based methods for the rapid, cost-efficient and high throughput assessment of toxicities of large numbers of environmental chemicals using in vitro reporter gene assays that measure the activation of key molecular stress pathways. The major molecular stress pathways are highly-conserved, well-characterized signaling pathways, triggered in response to environmental insult at doses significantly lower than those producing apical endpoints such as apoptosis. We have constructed and characterized an ensemble of reporter gene assays using promoter regions from canonical stress-responsive genes such as heme oxygenase I (HO) and heat shock protein 70 (hsp70) as well as multimerized synthetic elements that measure activation of stress-response factors such as Nuclear Factor kappa B (NFkB) and Nuclear Factor E2-related factor (Nrf2). The response of this assay ensemble is being used to obtain 'toxicological fingerprints' of compounds. To enable the delivery of reporters into cells of choice, these reporters were packaged into lentiviral vectors that allow for the rapid and efficient generation of stable reporter cell lines. We will present the results comparing the fingerprints of sets of related compounds, such as metals and persistent chemicals, in human cell lines of different tissue origins to evaluate the utility of the ensemble approach as a predictor of toxicity that may also permit the inference of the mechanistic basis of toxicity. (This abstract does not necessarily reflect EPA policy).

1427 EVALUATION OF ESTROGENIC ACTIVITY OF PHTHALATE ESTERS BY DNA MICROARRAY ANALYSIS.

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Phthalates are widely used as plasticizers, and are potential contaminants in water and the environments mostly either as di-ester or mono-ester complexes. Their structural similarity with natural estrogen suggests that they might have effect on reproductive organs and cancer development. Here, we used a DNA microarray containing estrogen responsive genes (EstrArray) to examine gene expression profiles in MCF-7 cells treated with 10 μM of butylbenzyl phthalate (BBP), dibutyl phthalate (DBP), diethyl phthalate (DEP) or diisopropyl phthalate (DIP), along with natural estrogen 17β-estradiol (E2, 10 nM). The profiles for phthalate esters and E2 were examined by correlation analysis using correlation-coefficients (R-values) and cluster analysis to show how close they are to each other and to E2. We found that BBP showed the highest correlation with E2 (R=0.85), while DEP and DIP showed moderate R values (R=0.52 and R=0.49, respectively). DBP exhibited the lowest (but still significant) correlation with E2 (R=0.36). Furthermore, among the pairs of chemicals, DEP-DIP and DIP-DBP showed very high correlations (R=0.90 and R=0.80, respectively), while the other pairs showed moderate relationships, which reflected how structurally close they are to each other. The analysis of six functional groups of genes (enzymes, signaling, proliferation, transcription, transport and others) indicated that the genes belonging to enzymes and transcription and the genes with other functions showed common responses to phthalate esters and E2. While the effect of BBP was similar to that of E2, the other phthalate esters clearly showed different types of effects. These results indicate that the structure of estrogenic chemicals is strongly related to their estrogenic activity and can be evaluated by appropriate grouping of the responsive genes by focused microarray analysis.

1428 FULL-GENOME ANALYSIS OF ALTERNATIVE SPLICING IN MOUSE LIVER AFTER HEPATOTOXICANT EXPOSURE.

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Alternative splicing plays a role in determining gene function and protein diversity. We have employed whole genome exon profiling using Affymetrix Mouse Exon 1.0 ST arrays to understand the significance of alternative splicing on a genome-wide scale in response to multiple toxicants. Mice were exposed to three hepatotoxicants including PFOA (perfluorooctanoic acid), propiconazole (conazole-triazole), and phenobarbital. Alternative splicing and gene expression were evaluated from the RNA isolated from the livers of treated and control animals. Preliminary analysis of transcript changes after PFOA exposure using a mixed model, nested analysis of variance (p-val < 0.001) and a FDR at 0.001 with Biotique's X-Ray software, suggested alternative exon usage for 600 transcript clusters. Ontological categorization of these genes indicated G-protein coupled receptor protein signaling pathway, cell surface receptor linked signal transduction, cellular lipid metabolism, generation of precursor metabolites and energy, lipid metabolism, carboxylic acid metabolism, organic acid metabolism, electron transport and fatty acid metabolism to be significantly altered. These categories of genes were also those that exhibited consistent gene expression changes in our historical data. We will use information about changes in alternative splicing to predict comprehensive changes in the structure of the proteome after chemical exposure, to identify new biomarkers of exposure, and to understand chemical mode of action. (This abstract does not necessarily reflect US EPA policy).

1429 NON-LINEAR LOW DOSE RESPONSE IN METHYL METHANESULFONATE (MMS)-TREATED RATS AS MEASURED BY MULTIPLE ENDPOINTS.

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The existence of thresholds for DNA reactive agents is a contentious topic. Using the well-characterized alkylating agent MMS, the relationship between dose (administered, systemic, and target), changes in liver transcriptome profile, and genotoxicity (peripheral blood micronuclei) were evaluated in male Fischer 344 rats. Groups of 6 rats were administered 0 (DMSO vehicle), 0.5, 1, 5, 25, or 50 mg MMS/kg bw/day (mkd) via oral gavage for 4 consecutive days. Systemic and liver doses were defined as hemoglobin (Hb) and hepatic DNA adduct levels, respectively. N-terminal methyl (Me)-Hb adducts were quantified as signature peptides using HPLC/ESI-MS/MS and demonstrated linear dose-response. Analysis of hepatic DNA for O^6- and N7-methylguanine (MeG) adducts as performed by LC/pESI-MS/MS identified N7-MeG adducts as NQ (not quantifiable; >0.8 /10^6 nt and <2.0/10^6 nt), NQ, 2.89, 9.30, 31.8, and 67.9/10^6 nt for control, 0.5, 1, 5, 25, and 50 mkd, respectively; O^6-MeG adducts were not quantifiable (<2.0/10^6 nt) at any dose. Gene expression from hepatic RNA was evaluated using an Agilent complete rat genome 4x44k array using one-color labeling and spike-in controls. Under the experimental conditions used, altered expression levels were observed only in 25 and 50 mkd group rats when compared to controls with 20 and 121 genes, respectively. Genotoxicity data collected from the same treated animals had a NOEL of 5 mkd and elevated MN frequency at 25 and 50 mkd ($p<0.05$). Thus, while MMS was systemically available in a linear dose-related manner, there were clear thresholds for the induction of DNA adducts, gene expression changes, and genotoxic changes. Collectively, these results provide evidence for the existence of thresholds for biologically significant events from DNA-reactive agents.

1430 ACRYLAMIDE AND GLYCIDAMIDE-INDUCED MITOCHONDRIAL GENE EXPRESSION PROFILES IN BIG BLUE TRANSGENIC MICE.

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The recent discovery of acrylamide (AA), a probable human carcinogen, in a variety of fried and baked foods has drawn attention to its genotoxicity and carcinogenicity. Evidence suggests that glycidamide (GA), the epoxide metabolite of AA, is responsible for the genotoxic effects of AA. Recently, we reported that both AA and GA are mutagenic in the liver of transgenic mice and that they altered significantly the expression profiles of genes involved in xenobiotic metabolism by cytochrome P450, glutathione metabolism, and xenobiotic signaling metabolism. In this study, we have also examined liver tissues for AA and GA-induced alterations in mitochondrial gene expression using a newly developed and validated mouse MitoChip containing 542 mitochondrial genes. Seven weeks-old, male mice were treated with 0, 500 or 600 mg/l AA or GA dissolved in drinking water, respectively, for up to 4 weeks. Weekly body weight, food and daily water consumption were monitored. Mitochondrial microarray analysis showed a significant change in expression level of 76 mitochondrial genes with a false discovery rate of less than 0.05. However, GA did not seem to influence transcriptional level of mitochondrial genes. An interesting finding was the AA-mediated significant decline in transcriptional level of several 3-beta hydroxysteroid dehydrogenases that are involved in steroid biosynthesis. In addition, genes associated with complexes I, III, IV and V of oxidative phosphorylation were differentially expressed. The observed significant down regulation in expression of hydroxysteroid dehydrogenases suggests that AA and GA, in addition to being genotoxic, may affect the endocrine function. These results provide further insight into the mitochondrial toxicogenomic mechanisms of AA and GA exposures in mice, which may be useful for human risk assessment.

1431 GENE EXPRESSION CHANGES IN BLOOD LEUKOCYTES INDICATIVE OF D-SERINE EXPOSURE.

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D-serine is a commonly used model nephrotoxin that selectively causes acute necrosis of the proximal straight tubules. We have previously investigated differential genes expression in the kidney resulting from d-serine exposure using a rat model (male F-344 rats treated with a single dose of d-serine ranging from 5 - 500 mg/kg and sacrificed 96 h post-treatment) and identified perturbation of a large number of pathways. In this study, we studied gene expression changes in the blood leukocytes isolated from the same animals using DNA microarrays followed by data analysis using t-test ($p<0.05$ over two consecutive doses), ANOVA (with Bonferroni correction) and Significance Analysis of Microarrays (SAM). Genes identified as differentially expressed in at least two of these methods were pooled and analyzed using self organizing map (SOM) resulting in two clusters of 110 and 151 up- and down-regulated genes respectively. Gene ontology search revealed that pathways implicated in immune response and hematopoietic cell lineages are up-regulated as a result of d-serine treatment. Also up-regulated are the pathways related to RNA processing, splicing and transport, oxidative stress and protein folding. Although there seems to be minimal overlapping in the final gene list between the kidney and blood leukocytes, perturbation of pathways including RNA processing, splicing and transport, oxidative stress response and protein transport was observed in both cases. Preliminary result of hierarchical clustering analysis, using the 13 genes that were identified in all three statistical methods with a fold-change > 3, revealed that control animals can be readily separated from the clusters of the low-dose and high-dose groups. This result suggests that gene expression changes in blood leukocytes might serve as surrogate markers for d- serine exposure and nephrotoxicity.

1432 APPLICATION OF *IN VITRO* TOXICOGENOMICS DURING INTERMEDIATE STAGES OF COMPOUND PROGRESSION.

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In vitro toxicology assays can be useful tools for rank ordering and selecting compounds for further in vivo evaluations or for structure-toxicity relationship studies. New tools, particularly in vitro toxicogenomics, have the advantage of allowing for the simultaneous evaluation of multiple toxicity endpoints, in contrast to conventional assays. Due to their high content, they represent a unique approach to elucidate mechanisms of toxicity, to identify novel biomarkers for the identification of toxic endpoints, and to predict potential safety liabilities. However, questions have arisen regarding at which stage in vitro toxicology assays should be employed. Often, compounds are optimized for efficacy before the compound is evaluated for potential adverse effects. This is based on the assumption that until efficacy is optimized there is little value in performing toxicology studies, since earlier versions of a compound may be structurally significantly different than the final product. However, definitive experiments have not been done to determine if precursor compounds display similar effects as more optimized compounds. Using toxicogenomics and other cell-based assays, we evaluated three different drug candidates and their synthetic precursors to identify whether early compound leads and more optimized compounds yield similar toxicology endpoints. Overall, reasonable consistency for the in vitro assays was observed between earlier lead compounds and the final optimized products. The information generated from these studies will be useful for positioning the use of in vitro toxicology assays, and ultimately will lead to a more rational selection of compounds in discovery.

1433 MICROARRAY ANALYSIS OF MOUSE LIVER TRANSCRIPTS FOLLOWING LOW DOSE ARSENIC/DEXAMETHASONE EXPOSURE.

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Arsenic (As) is an environmental toxicant of great concern worldwide. Chronic exposure has been associated with increased risk of cancer and a growing list of other serious diseases, but the underlying mechanism(s) for these various health effects remains unclear. In this study, mice (C57BL/6J, 9 wk) were exposed for 5 wk to a low-As AIN-76A diet with 0, 10 or 100 ppb As in drinking water, and some mice were also treated acutely (8 hr) with As (1 mg/kg ip) and/or dexamethasone (Dex, 1 mg/kg ip). Affymetrix 430 2.0 whole genome microarrays were used to assess global gene expression changes in the liver and RankProd statistics were used to determine regulated genes. Ingenuity software was used to identify pathways of interest. The common altered pathways after either acute or chronic As exposure included lipid, carbohydrate, amino acid and xenobiotic metabolism, cell growth, oxidative stress, immune response and endocrine signaling. There were clear effects and differences between the 10 and 100 ppb chronic exposures. Lipid metab-

olism pathways were strongly implicated in chronic 100 ppb exposure while various endocrine signaling pathways were more prevalent at the 10 ppb As exposure. Key genes of interest were confirmed by real-time RT-PCR, including GREB1 (Gene Regulated by Estrogen in Breast cancer). GREB1 is linked to hormone-dependent cancer growth and was strongly affected by acute treatments, being induced by Dex while As repressed the Dex induction. This novel finding was followed by studies using H4IIE rat hepatoma cells, where, interestingly, GREB1 was repressed by Dex whereas As muted the Dex repression. (NIH-NIEHS SBRP P42 ES07373, P2).

1434 ANTI-INFLAMMATORY EFFECT OF SAPONINS ISOLATED FROM THE ROOTS OFPLATYCODON GRANDIFLORUM IN A MOUSE MODEL OF ALLERGIC LUNG INFLAMMATION.

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Asthma is a chronic respiratory disease, the incidence of which is increasing globally. In this study, we investigated protective effect of the saponins isolated from the roots of Platycodon grandiflorum (Changkil saponins, CKS) on airway inflammation in a mouse asthma model immunized and challenged with ovalbumin (OVA). Mice sensitized and challenged with OVA had increased amounts of T-helper cell type 1 and 2 (Th1 and Th2) cytokines in bronchoalveolar lavage (BAL) fluid. Administration of CKS before OVA challenge resulted in a significant inhibition of all asthmatic reactions. Therefore, to determine if CKS regulate some parameter associated with asthma, their parameters such as OVA-specific IgE, interleukin (IL)-4, IL-5, IL-13, interferon-g, tumor necrosis factor-a and monocyte chemotactic protein-1were measured in BAL fluid by ELISA. As the result, all parameters were attenuated in a dose-dependant manner by the administration of CKS. These results suggest that CKS may attenuate the development of airway inflammation by reducing Th1 and Th2 cytokines.

1435 SAPONINS DERIVED FROM ROOTS OF PLATYCODON GRANDIFLORUM INHIBITS INFLAMMATORY CYTOKINE EXPRESSION IN RAT MAST CELLS.

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Mast cells are central regulators of allergic inflammation through production of various chemical mediators and cytokines. Antigen (Ag)-stimulated mast cells induce synthesis and production of inflammatory cytokines including tumor necrosis factor (TNF)-alpha and interleukin (IL)-6. In this study, we investigated the effects of Changkil saponin fraction (CKS) derived from roots of Platycodon grandiflorum on the inflammatory cytokine expression in Ag-stimulated rat basophilic leukemia (RBL)-2H3 mast cells. CKS had potent antioxidative effects such as superoxide radical scavenging activity and the inhibition of ROS production. CKS reduced the Ag-induced beta-hexosaminidase and histamine release in RBL-2H3 cells. In addition to, CKS inhibited Ag-induced TNF-alpha and IL-6 production and their expression. These findings suggested that CKS has anti-inflammatory effects and may be useful allergic disease such as atopic dermatitis.

1436 UP-REGULATION OF CYCLOOXYGENASE-2 EXPRESSION BY SERINE PROTEASE, SUBTILISIN, IN MACROPHAGES.

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Subtilisin is produced by various Bacillus subtilis strains and other Bacillus species. Subtilisin is serine proteases that evolved independently of chymotrypsin. Proteases, specifically serine proteases, play also a major role in inflammation. In the present study, we investigated the effects of subtilisin on the cyclooxygenase-2 (COX-2) gene expression in the murine macrophage Raw 264.7 cells. Subtilisin significantly increased the prostaglandin E2 production, a major COX-2 metabolite. In addition to, COX-2 mRNA level were markedly induced in the treatment of subtilisin in a dose dependent manner. Transient transfection experiments also showed that COX-2 promoter activities were increased by subtilisin. Overall, this study provides evidence showing that subtilisin has inflammatory potential that is previously unrecognized immunomodulating activity.

1437 ALCALASE-MEDIATED GENE EXPRESSION PROFILE CHANGE IN HUMAN AIRWAY EPITHELIAL CELL LINE A549 GROWN IN AN AIR/LIQUID INTERFACE.

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Type I respiratory allergy is a Th2 dominated immune response with development of allergen-specific IgE. The initial interaction of an allergen with airway epithelial cells may be crucial in determining whether a Th2-dominated (vs Th1-dominated) immune response will be generated. In previous in vitro studies we showed that Alcalase® (Bacillus serine protease and known occupational respiratory allergen) induced a dose-dependent production of the inflammatory cytokine, MCSF, from human airway type II-like bronchial epithelial cell line A549 grown in an air/liquid interface. In this study using Affymetrix Human U133 Plus 2.0 GeneChip®, gene expression profiles of A549 cells treated with 10 or 100 ug/ml Alcalase at two time points, 6 h and 24 h, were compared to profiles in untreated A549 cells. Following 100 ug/ml Alcalase treatment, the expression level of MCSF gene was up regulated 2- fold at 6 h, and 1.9-fold at 24 h. Using filtering criteria of a 1.5 or greater fold-change in expression and suitable control of the false discovery rate ($p < 0.05$), 89 out of 240 annotated interleukin, interferon and tissue necrosis factor related probe sets were differentially expressed. Using filtering criteria of a 1.5 or greater fold-change in expression and suitable control of the false discovery rate ($p < 10\text{-}6$) in dose related responses, the Gene Ontology and KEGG pathway terms associated with 457 genes were examined. The ontology term analysis showed that Alcalase treatment led to expression level changes for genes involved in the immune response, cell cycle, energy metabolism and tight junction function. The analysis of KEGG pathway terms identified the Jak-Stat signaling pathway, cytokine-cytokine receptor interactions and cell cycle genes as significantly over represented. Taken together we are building an understanding of how a potent type 1 allergen affects human airway epithelial cells.

1438 A DRAINING LYMPH NODE ASSAY (DLNA) AS A POTENTIAL FIRST TIER FOR ASSESSING THE SENSITIZING POTENTIAL OF PROTEINS.

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There is a need for a simple and predictive animal model to identify the respiratory sensitization potential of (novel) proteins. The present study examined the use of a DLNA approach, employing several routes of exposure, as a potential starting point for assessing protein sensitization potential. Consistent with the standard local lymph node assay, female BALB/c mice were dosed dermally (DER), intranasally (IN) or by oropharyngeal aspiration (OP) on days 1, 2 and 3, and proliferation in the relevant draining lymph nodes was measured on day 6. For each route, the auricular, superficial cervical and tracheobronchial (TBLN) lymph nodes were evaluated following treatment with Subtilisin Carlsberg (SUB; known respiratory sensitizer), ovalbumin (OVA; potent food allergen), β-lactoglobulin (BLG; moderate food allergen), and keyhole limpet hemocyanin (KLH; strong immunogen with no reports of respiratory sensitization). Initial studies with OVA indicated that DER administration did not stimulate lymph node proliferation. Responses in the TBLN were most dramatic (stimulation indices up to 100) and reproducible for both the IN and OP routes. In a comparative experiment, all proteins induced lymph node proliferation with a rank order potency of SUB > KLH > OVA > BLG. The influence of the endotoxin content on lymph node proliferation was determined to be minimal, and did not impact the rank order potency. Molecular characterization of the TBLN at an equipotent proliferative dose was conducted for select gene transcripts (Gzmb, IL4, Akr1c18, IL21) chosen based on research examining chemical sensitizers. Expression profiles differed among the four proteins, but the relevance of these responses was not clear and they did not further discriminate their allergic potential. These data illustrate the potential utility of examining the DLN response to proteins, but indicate additional endpoints and/or optimized dosing and temporal examinations are required to assess their allergenic potential by this approach.

1439 IMMUNOGLOBULIN E IN ASTHMATIC HUMAN SERA IS REACTIVE AGAINST MOLD EXTRACTS.

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Molds have been associated with various health effects including asthma, but their role in induction of asthma is unclear. However, the presence of mold-specific IgE indicates their capacity to induce allergic responses and possibly exacerbate asthma

symptoms. This study was undertaken to 1) provide insight into the prevalence of human IgE-reactivity to mold proteins, 2) identify the target mold protein(s) and 3) compare human IgE target proteins with mouse IgE-reactive protein profiles. Human sera were collected from thirty-three individuals (11 adults and 22 children) with persistent asthma, living near Chapel Hill, NC. The sera were screened for IgE-reactivity against selected indoor mold extracts by chemiluminescent dot blot analysis. Tested protein extracts include Penicillium chrysogenum, Metarhizium anisopliae, Stachybotrys chartarum, Cladosporium cladosporiodes, mold mix 1 and 2 (Greer lab, Lenoir, NC), and house dust mite. The resulting chemiluminescent signal was quantified by densitometry. Strongly reactive sera were chosen to identify the IgE-binding proteins by Western blot analysis. These protein profiles were compared to those identified with extract-specific mouse serum IgE. The dot blot screening demonstrated variability among individuals and within an individual's level of IgE-responsiveness to the various mold extracts. IgE-reactive protein profiles from human sera were similar to those detected using mouse serum. In summary, most asthmatics in this study had IgE-reactivity against at least one mold. Additionally, both the mouse and human immune systems are generally reactive to the same mold proteins. (This abstract does not reflect EPA policy.)

1440 IMMUNOLOGIC SKEWEDNESS TOWARD TYPE-2 RESPONSE PRONE TO RESPIRATORY ALLERGY IN PUBLIC SERVICE FACILITY EMPLOYEES.

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The present study was to evaluate the immunologic parameters including genetic polymorphisms reflecting susceptibility to respiratory allergic diseases in the employees working at public service facilities. 57 workers were volunteered from 7 child care, 7 elderly care, 8 transportation facilities, and 4 supermarkets. 17 control subjects were age and sex-matched. Skin prick test against 25 aeroallergens and metacholine bronchial challenge test were carried out to all participants (IRB approval by Eulji University School of Medicine). Plasma levels of IgE and histamine were determined. Genetic variations in IL4, IL13, and IL4R were analyzed by RFLP. Level of IL-4 and IFNγ production from PBMC stimulated with a polyclonal stimulator was determined. Aeroallergen positive skin reaction was observed at 53% of the employees with no difference from the control. House dust mite was the most prevalent allergen (38%) followed by fungi (22%), weed pollen or animal fur (11% each), cockroach (8%) with no difference among the groups. The employees irrespective of prick test positivity demonstrated higher histamine level and lower FEV1/FVC % than the control. The relative increase in IL-4 to IFNγ level in the employees (IL-4:IFNγ ratio=22) was evident in comparison with that of the control (the ratio=3). Even though no symptoms or pulmonary functional abnormalities on respiratory allergic diseases, plasma IgE level and eosinophils % in peripheral blood were higher in the prick test positive than the negative employees. But we could not observe any significant difference in the IL-4 (C589T), IL-13 (Arg130 Gln), or IL-4R(Gln576Arg and Ile576Val) genetic polymorphism between the aeroallergen positive and the negative subjects. This study substantiate that upregulation of Th2 reactivity in the employees could be implicated in susceptibility to various allergic diseases in the absence of genetic influence.

1441 MIXED AIR POLLUTION, ALLERGY AND INFLAMMATION.

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Air pollution is suggested as a contributing factor for increased prevalence of airway allergy, and air pollution is shown to aggravate existing asthma and allergy. Diesel exhaust particles (DEP) are one component that is shown to amplify an allergic immune response. The aim of this work was to investigate if nitrogen dioxide (NO_2), that is another important constituent in automotive air pollution, influences on the allergic immune response and upon the adjuvant effect of DEP, i.e. a combined effect of NO_2 and DEP. Balbc/A mice were exposed intranasally at three consecutive days to the model allergen ovalbumin (10 μg per animal per day) with or without DEP (67 μg per animal per day) present, followed by inhalation of 5 or 25 ppm NO_2 for four hours. 18 hours after the last exposure the lungs of half of the animals were washed with saline and the broncho-alveolar lavage (BAL) fluid was analyzed for TNFα, total protein and by differential counting of cells. After three weeks the mice were booster immunized three times with OVA (10 μg per animal per day), and after four weeks blood was sampled by heart puncture under CO_2 anesthesia. 5 ppm of NO_2 seemed to induce increased amounts of TNFα, whereas DEP increased total protein in the BAL fluid, in the presence or absence of OVA. No significant changes were seen for cell numbers. Earlier observations of the IgE adjuvant effect of DEP were confirmed. 5 ppm NO_2 together with OVA and DEP did not additionally increase allergen specific IgE. Neither was IgG2a serum concentrations increased. 25 ppm NO_2 induced increased amounts of protein and TNFα in all groups, both buffer control and those exposed to OVA with or without DEP present. The low dose of NO_2 may induce inflammation, but had no effect on allergy. However, the high dose induced inflammation and lung damage seen as leakage of proteins from blood into the lungs. The effect of 25 ppm NO_2 on the production of IgE is at present to be analyzed.

1442 AN ANIMAL MODEL OF PLATINUM (PT) HYPERSENSITIVITY.

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Exposure to Pt salts has been associated with occupational asthma. Pt, the most active component and widely used metal in catalytic converters, is released in automobile exhaust and is a proposed diesel fuel additive. Thus, with the potential for widespread environmental distribution, the risk for asthma in the general population is a concern. We describe here a mouse model of Pt sensitization that may be useful for studying risks associated with Pt allergy. Mice were sensitized dermally with 100 μl 1% ammonium hexachloroplatinate (AHCP) in DMSO to the shaved back on days 0, 5 and 19, and 25 μl to each ear on days 10, 11 and 12. Unsensitized mice received vehicle. On day 24, sensitized mice were challenged intranasally (i.n.) with 0, 1, 3, 10 or 30 μg AHCP in saline with or without 50 μg of diesel exhaust particles (DEP). Unsensitized mice were challenged i.n. with 0 or 30 μg AHCP with or without DEP. Airway responses were assessed by whole body plethysmography (WBP) before and immediately after i.n. dosing. On day 26 reactivity to methacholine aerosol was assessed by WBP. Mice were then sacrificed and serum and bronchoalveolar lavage fluid (BAL) collected. A significant increase in total serum IgE occurred in all sensitized mice. A dose dependent increase in immediate airway responses (IAR) was observed in AHCP sensitized and challenged mice. Unsensitized mice challenged with 30 μg AHCP had an increased IAR relative to vehicle mice, but significantly lower than sensitized mice challenged with 30 μg AHCP. Modest dose dependent increases in MCH responsiveness occurred, but without corresponding changes in BAL cell influx. Exposure to DEP had no effect on any parameters. The data show that mice sensitized to Pt salts via the dermal route respond to respiratory exposures. This model may be useful for assessing cross reactivity between different forms of Pt salts, further exploration of adjuvant effects of DEP, developing dose responses, and screening of DEP derived from burning fuels with Pt additives. (This abstract does not reflect EPA policy).

1443 IDENTIFICATION OF RESPIRATORY SENSITIZING CHEMICALS OF LOW MOLECULAR WEIGHT USING BRONCHOALVEOLAR LAVAGE FLUID CYTOKINES AND SERUM IGE.

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It has been reported that exposure to some kinds of low molecular chemicals induced respiratory sensitization in workplace. The allergic reaction, especially asthma, is a serious problem for workers. However, there are no validated test methods to identify the respiratory sensitizing chemicals at present. Many animal studies show the advantage of measuring cytokines and serum IgE to identify the respiratory sensitizing chemicals. In this study, we used three respiratory sensitizers; dicyclohexylmethane-4,4'-diisocyanate (HMDI), diphenylmethane-4,4'-diisocyanate (MDI) and phthalic anhydride (PA), and two contact sensitizers; 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (oxazolone) and hexylcinnamaldehyde (HCA) to examine the effectiveness of the indicators mentioned above. Female BALB/c mice were dermally exposed with chemicals on the shaved flanks on days 0, 1, 6 and 7. Additional dermal exposure of chemicals was conducted to the dorsal surface of each ear on days 12, 13 and 14. On day 27, the treated mice were challenged by intratracheal instillation of chemicals. At 24 hours after the challenge, bronchoalveolar lavage fluid (BALF) samples and sera were collected. Cytokines in BALF were analyzed by multiplex assay (Bio-Plex) and the concentration of serum IgE was measured by ELISA. Ten cytokines were selected to measure based on the preliminary experiment. Mice exposed with respiratory sensitizers showed statistically significant increases in 2 to 6 BALF cytokines compared to each control group. Mice sensitized with HCA also showed statistically significant increases in 2 BALF cytokines. In contrast, oxazolone sensitized mice showed no increase in cytokines. Mice sensitized with HMDI, MDI, PA or oxazolone showed statistically significant increase in serum IgE levels. Mice sensitized with oxazolone showed lower serum

IgE levels than those sensitized with respiratory sensitizers. BALF cytokines and serum IgE levels could be the effective indicators for the identification of respiratory sensitizers.

1444 EVALUATION OF RESPIRATORY CHEMICAL ALLERGENS IN THE PEPTIDE REACTIVITY ASSAY.

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Chemical respiratory allergy is an adverse health effect of concern. Compared with the hundreds of low molecular weight chemicals implicated as skin sensitizers, far fewer are known to cause sensitisation of the respiratory tract. However, experience to date suggests that chemicals that cause respiratory allergy in humans elicit positive responses in animal tests for skin sensitization. In contrast to the case for chemical skin allergens, there are no well-validated or widely accepted available test methods for the identification of chemical respiratory sensitizers. Like contact allergens, respiratory chemical allergens or their metabolites, have electrophilic properties and need to react with proteins (usually thru nucleophilic amino acids) for the effective induction of sensitization. We do not understand why some chemicals preferably sensitize the respiratory tract while others are contact skin sensitizers. Using an in vitro peptide reactivity assay under development for screening skin chemical sensitizers, we tested several respiratory chemical sensitizers to see if we could identify any differences in how the two types of chemical sensitizers interact with nucleophile containing peptides. Chemicals were incubated with cysteine and lysine containing heptapetides and peptide depletion was assessed. Toluene 2, 4-diisocyanate, and diphenylmethane di-isocyanate showed moderate reactivity to both peptides while hexamethylene diisocyanate showed high reactivity to both peptides. Hexahydrophthalic anhydride and phthalic anhydride showed little reactivity to the cysteine peptide, trimellitic anhydride showed little reactivity to the lysine peptide while maleic anhydride showed high reactivity to both peptides. The low reactivity to peptides may be due to steric hindrance or high reactivity with water. We need to continue testing known respiratory chemical sensitizers to assess whether there is a pattern to their reactivity with peptides that may set them apart from chemical contact allergens.

1445 CARBON NANOTUBES ENHANCE ALLERGIC RESPONSES IN MICE.

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Particulate air pollution is associated with aggravation of asthma and allergy, and may also have contributed to the increased prevalence of asthma and allergy in industrialized countries. Various particles have been shown to increase the allergic immune response to allergens both in humans and animals. In mice, the adjuvant effect of particles on allergic responses has been demonstrated to increase with decreasing particle size, thus with increasing total particle surface area. This suggests that carbon nanotubes, which have a large surface-to-mass ratio, may be especially potent in enhancing the antibody responses to allergens. The scope of this study was to investigate whether single-walled carbon nanotubes (swCNT) and multi-walled carbon nanotubes (mwCNT) have the potency to enhance the production of allergen-specific IgE (hallmark molecule of allergy) and IgG2a. Balb/cA mice were exposed to three doses of the swCNT, mwCNT, as well as 14 nm carbon black (CB, Printex90) particles as a reference particle during sensitization with the allergen ovalbumin (OVA). Animals were sensitized by a single subcutaneous injection of OVA and particles into the footpad, and boosted by an OVA injection three weeks later. Five days after the OVA booster, blood samples were collected and OVA-specific IgE and IgG2a antibodies in serum were measured by ELISA. The swCNT and mwCNT increased the serum levels of OVA-specific IgE, but did not or only slightly increase the OVA-specific IgG2a levels. The mwCNT tended to be more potent than the swCNT, and seemed to enhance the IgE response to a similar degree as the CB particles. These hazard identification data suggest that exposure to carbon nanotubes may aggravate and enhance the development of allergic responses. Data from similar experiments in an intranasal model are currently being analyzed.

1446 ACTIVITY OF CHEMICAL RESPIRATORY SENSITIZERS IN A NON-STANDARD LOCAL LYMPH NODE ASSAY (LLNA).

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Although allergic sensitization of the respiratory tract induced by chemicals is not as common as skin sensitization, it is an important occupational health issue. Several experimental approaches have been proposed for the identification of chemical respiratory allergens but none of these has yet been validated formally or widely accepted. We have elected to explore their relationship with skin sensitizing chemicals. The activity of a series of chemicals known to cause respiratory allergy or immediate type hypersensitivity reactions in humans has been examined in the mouse LLNA. This method is based on the measurement of induced proliferative responses in draining lymph node cells as assessed by incorporation in vivo of radiolabelled thymidine. A positive response in the LLNA is defined as those materials that provoke a 3-fold or greater increase in proliferation compared with concurrent vehicle treated controls. For practical reasons, a nonstandard mouse (BALB/c) strain, not the recommended CBA strain, was used in these studies. This strain has been shown previously to perform similarly to the CBA strain. The chemical respiratory allergens used in these studies were Reactive Black 5, Reactive Yellow 39, trimellitic anhydride, phthalic anhydride, toluene diisocyanate, hexamethylene disocyanate and two uronium salts (tetramethyluronium hexafluorophosphate and tetramethyluronium tetrafluoroborate). All of the respiratory allergens tested were found to elicit relatively vigorous positive responses in the LLNA at one or more doses tested. The accuracy of the LLNA for contact allergens has been demonstrated previously with negative results recorded for 40 out of 42 nonallergens. We propose that chemicals that fail to elicit positive responses in the LLNA might be regarded as lacking the inherent potential to cause either contact sensitization or allergic sensitization of the respiratory tract.

1447 EVALUATION OF THE POTENTIAL AND STRENGTH OF SKIN SENSITIZATION USING *IN VITRO* SKIN SENSITIZATION TEST - HUMAN CELL LINE ACTVATION TEST (H-CLAT).

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We have developed the h-CLAT, an in vitro skin sensitization test using THP-1 cells (human monocytic leukemia cell line). This test is based on the augmentation of CD86 and/or CD54 expression in THP-1 cells following exposure to chemicals. We previously confirmed that the h-CLAT protocol is easy to transfer and that good inter-laboratory reproducibility was observed in an inter-laboratory study in Japanese, European, and US laboratories. In this study, we evaluated about 90 chemicals with different potentials of skin sensitization in the h-CLAT and compared our results with LLNA or human test data for the same chemicals. The accuracies of the h-CLAT vs. LLNA or human test data were about 85% or 80%, respectively. Most chemicals were evaluated correctly, but a few chemicals failed. The chemicals classified in the h-CLAT as "false-negative" were geraniol, phthalic anhydride and others, while the chemicals classified as "false-positive" were 1-bromobutane, diethylphthalate and others. The "false-negative" findings might be due to a lack of metabolic activity in THP-1 cells or a low solubility in test vehicle. Next, we calculated the estimated concentration to induce marker expression with an RFI=150 for CD86 (EC150) or 200 for CD54 (EC200) in the h-CLAT. These values were then compared with the LLNA EC3 values. Both RFI values have a good correlation with the LLNA EC3 values. We ranked the h-CLAT findings into 2 groups, "strong" and "weak", using the EC150 (CD86) value as the cut-off level. As a result, the "strong" group in h-CLAT had a high correlation with the extreme and strong groups of the LLNA, and the "weak" group in h-CLAT correlated with the moderate and weak groups of the LLNA. Our result suggests that the h-CLAT is a useful test for the prediction of skin sensitization potential and strength. Thus, the h-CLAT could be one of the key tests in a battery system that addresses each crucial mechanism of skin sensitization.

1448 INVESTIGATION OF FALSE-NEGATIVE CONTACT ALLERGENS IN THE IN VITRO SKIN SENSITIZATION TEST; HUMAN CELL LINE ACTIVATION TEST (H-CLAT).

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The h-CLAT is based on augmentation of CD86 and CD54 expression in THP-1 cells (human monocytic leukemia cell line) following exposure to allergens. The h-CLAT could be a useful *in vitro* method for predicting the skin sensitization potency of chemicals. However, there were some false-negative chemicals. In this study, we investigated the reasons for the false-negatives in order to clarify the applicability domain of the h-CLAT.

Geraniol is a fragrance terpene that has the potential to autoxidize on air exposure and form highly allergenic compounds. In the h-CLAT, fresh geraniol (from a new bottle) was negative while geraniol from an older opened bottle was positive. In the latter lot, the concentration of geraniol was decreased to 85% and partly transformed into citral. Citral is one of the oxidation products of geraniol and it was positive in the h-CLAT. Therefore, fresh geraniol was a false-negative due to the

lack of transformation by air-exposure under the test condition. To evaluate fragrance terpenes, called prehaptens, test chemicals might need to be pretreated by oxidation.
For abietic acid, fluorescence derived from the test chemical itself occurs under the flow cytometric analysis conditions in the h-CLAT. In order to assess possible effects on THP-1 cells, we measured CD86 and CD54 gene expression in THP-1 cells following exposure to abietic acid. The quantitative RT-PCR analysis showed that abietic acid significantly induced CD54 mRNA expression. Therefore, although abietic acid was negative in the h-CLAT, immunologically-relevant physiological changes occur in THP-1 cells following exposure to abietic acid. For fluorescent chemicals with negative findings in the h-CLAT, consideration of the possible interference of intrinsic chemical fluorescence needs to be taken.
Further evaluations of the false-negative allergens revealed chemical specific conditions that lead to the false-negative results in the h-CLAT. Through these evaluations, we are sure that the applicability domain of the h-CLAT could be clarified.

1449 EXAMINATION OF A HUMAN CELL LINE ACTIVATION TEST (H-CLAT) FOR PREDICTING SKIN SENSITIZATION POTENTIAL: OUTCOME OF THE THIRD RING TRIAL.

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Evaluation of changes in cell surface marker expression induced in dendritic cells (DC) or DC-surrogate cell lines following exposure to contact allergens represents one approach for the development of non-animal test methods for skin sensitization. The human Cell Line Activation Test (h-CLAT) examines by flow cytometry the level of expression of CD86 and CD54 on the surface of THP-1 cells, a human monocytic leukemia cell line, following 24 hours of chemical exposure. To date, the h-CLAT has been evaluated by five independent laboratories in a series of three ring trials coordinated by the European Cosmetic, Toiletry and Perfumery Association (COLIPA). The results of the first and second ring trials demonstrated that the protocol was transferable and identified areas for protocol and prediction model improvement. A third ring trial using the modified procedures and prediction model was completed in early 2007. The sensitization potential of eight chemicals was evaluated and included five sensitizers, one non-sensitizer and two irritants. All laboratories correctly classified the sensitizing capacity of six of the eight materials. Hexyl cinnamaldehyde was mis-classified as a non sensitizer by all labs and methylchloroisothiazolinone/methylisothiazolinone was misclassified by three of the five labs. As a result of this third ring trial, opportunities for further refinement of the h-CLAT protocol and prediction model were identified. While additional work is needed, the feasibility of utilizing cell lines as surrogate DC in development of in vitro skin sensitization methods shows promise

1450 INTRA- AND INTERLABORATORY EVALUATION OF THE LLNA-DA METHOD: A NON-RI MODIFIED LLNA IN WHICH ATP CONTENT IS USED AS THE ENDPOINT.

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Background: The murine local lymph node assay (LLNA) is a well-established method for assessing the skin-sensitizing potential of chemicals. We have developed a modified non-radioisotopic (RI) LLNA method, termed LLNA-DA (LLNA modified by Daicel based on ATP content), in which ATP content is used as the endpoint of cell proliferation instead of [^{3}H]thymidine incorporation.
Method: Groups of female CBA/JNCrlj mice were treated with a topical application of the test chemicals or a vehicle control to the dorsum of both ears on days 1, 2, 3, and 7. One hour before each application, the dorsum of each ear was pretreated with 1% sodium lauryl sufate (SLS) solution. On day 8, the auricular lymph nodes were excised, and single-cell suspensions were immediately prepared from each animal. The ATP content was measured by the luciferin-luciferase method after appropriate dilution. A stimulation index (SI) relative to the concurrent vehicle control was derived, and an SI of 3 was set as the cut-off value.
Results: We tested 31 well-known chemicals for the intralaboratory studies and clear dose-dependent relationships were observed for the positive chemicals. Modification of the dosing schedule and pretreatment with SLS enhanced the SI value, as a result of which, the EC3 values obtained by LLNA-DA were adjusted to those of the traditional LLNA. The accuracy of LLNA-DA vs. LLNA, guinea pig tests, and human tests were 93%(28/30), 80%(20/25), and 79%(15/19), respectively.
Two rounds of interlaboratory validation studies using this method, in which the result of the first round study was previously presented at the SOT's 46th annual meeting, have been conducted. In total, including the second round study, 14 chemicals in 17 laboratories were tested; acceptable small intra- and interlaboratory variations in SI values were obtained.
Conclusions: These investigations suggest that LLNA-DA has both adequate relevance and reliability, and will be a useful alternative method that has a non-RI endpoint.

1451 INTER-LABORATORY VALIDATION OF A NON-RADIOISOTOPE VERSION OF THE LLNA*.

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The local lymph node assay (LLNA) is a stand-alone test method for assessing the skin sensitization potential of chemicals. The traditional method uses tritiated thymidine to measure sensitizer-induced cell proliferation in the auricular lymph nodes of the mouse. To eliminate the need for a radioisotope in this assay, which significantly limits its widespread use, we have evaluated a non-radioactive LLNA protocol where the only modification is the use of 5-bromo-2'-deoxyuridine (BrdU) and not tritiated thymidine to assess cell proliferation in lymph nodes. Our first assessment of inter-lab reproducibility and relevance, conducted in 2006, indicated excessive variability among the 9 labs in correctly classifying coded chemicals as sensitizers/non-sensitizers. To make the assay more reproducible and relevant, we revised the protocol by more clearly describing the process for obtaining a lymph node cell suspension. Using this revised protocol, we tested 10 chemicals (4 strong sensitizers/3 weak sensitizers/3 non-sensitizers) among 7 labs with 6 chemicals being tested in each lab. Three of the chemicals were tested by all 7 labs, with 3 other chemicals tested in 3 labs each. Using this revised protocol, more consistent results were obtained among the 7 labs. This approach is expected to generate a highly reproducible, non-radioisotope LLNA protocol for international regulatory use.
*Takeyoshi M., et al., Development of non-radio isotopic endpoint of murine local lymph node assay based on 5-bromo-2'-deoxyuridine (BrdU) incorporation, Toxicology Letters, 119(2001) 203-208.

1452 TESTING WATER SOLUBLE ALLERGENS *VIA* THE EAR CANAL IN THE LOCAL LYMPH NODE ASSAY.

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The local lymph node assay (LLNA) is the preferred animal model for the identification of chemicals with the potential to induce skin sensitization. However, the model is not suitable for water soluble materials. We therefore assessed the application of water soluble materials into the ear canal instead of to the dorsum of the ears, while otherwise following the OECD protocol 429 with groups of CBA/Ca mice (n=5). We also evaluated whether this modified LLNA could discriminate between contact and respiratory sensitizers using IFN-γ, IL-4 and IL-13 cytokine endpoints after incubation of the cells from the auricular lymph node. The following known sensitizers were tested: the skin sensitizers 2-mercaptobenzothiazole, being slightly water soluble, and the traditional positive control hexyl cinnamic aldehyde in acetone olive oil, as this chemical is insoluble in water; and the respiratory sensitizers, the slightly water soluble chemical ammonium hexa chloroplatinate, and two industrial enzymes, a protease from B. subtilis and a phytase from A. oryzae. The vehicles were the negative controls.
The results demonstrated that application into the ear canal is a useful application procedure for water soluble materials using an aqueous vehicle. The dosing procedure is easy, it can be performed by one person only and the animals do not have to be single housed. The animals are not affected by the dosing of aqueous solutions and the application has the advantage of being more accurate than dosing the dorsum. The direct stimulation index read-out of the present LLNA studies identified the protein-based preparations as skin sensitizers. Enzyme proteins are, however, known to be human respiratory allergens, and not human skin sensitizers, and therefore a model to discriminate between the two types of sensitizers is highly needed. The results from the present cytokine profiling showed that this model may be able to discriminate the predominant T-helper 2 cell activation of water soluble respiratory protein sensitizers from the T-helper 1 cell activation after exposure to chemical contact allergens.

1453 SENSITIZATION POTENTIAL OF VETIVERYL ACETATE EVALUATED USING THE LOCAL LYMPH NODE ASSAY.

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Vetiveryl acetate is a fragrance material prepared by the acetylation of vetiver oil. Three possible production processes yield complex mixtures that vary in composition based on the geographic and botanic origins of the starting material. These production processes result in four distinct commercial qualities of vetiveryl acetate that are used in perfumery. A sample of each commercial quality was tested in the Local Lymph Node Assay (LLNA) to characterize its sensitization potential and to compare the potency of each. The LLNAs were conducted according to the methods described in OECD Guideline 429. Groups of five CBA/J female mice were utilized throughout the study. Each material was tested at five dose levels ranging from 1% to 25% w/v in 1:3 ethanol:diethyl phthalate. The Stimulation Index (SI) values were calculated for each dose level based on pooled lymph nodes, and an SI of 3 or more was considered a positive response. Linear interpolation of the dose response data from each LLNA was used to derive an estimated concentration (EC3) required to elicit an SI value of 3. The EC3 value was then taken as a measure of relative potency and used to compare the sensitization potential between each quality. All four samples of vetiveryl acetate can be regarded as potential sensitizers because the tests resulted in SI values greater than 3. EC3 values ranged from 9.3% to 13.3% (2317 $\mu g/cm^2$ to 3325 $\mu g/cm^2$). The study demonstrated that there was little difference in the sensitization potential between each quality, and the materials are considered weak dermal sensitizers. The data from this study will be useful in guiding the direction of future human studies to confirm a No Expected Sensitization Induction Level (NESIL) and provide information to a Quantitative Risk Assessment (QRA) for these materials.

1454 IMPACT OF OLIVE OIL AND DMSO AS VEHICLES ON MURINE LOCAL LYMPH NODE ASSAY (LLNA) ESTIMATED BY TWO SETS OF ENDPOINTS.

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The LLNA is a useful method in assessing the skin sensitizing potency of chemicals. However, the vehicles used in the test may have an impact on the accuracy of skin sensitizer identification. Olive oil (OO), dimethyl sulphoxide (DMSO) and mixtures thereof were assayed to estimate the effect of these vehicles on LLNA response potency and to evaluate the reliability of two different sets of endpoints. The methods were based on the OECD Guideline No. 429. Groups of five female CBA/CaOlaHsd mice were topically treated with olive oil, OO-DMSO at 75%, 50%, or 25%, or neat DMSO on three consecutive days. Five negative control mice (NCG) remained untreated. Five days after the first topical application, the draining lymph node cell (LNC) activation was detected individually using (a) the classic endpoint (dpm/mouse), and (b) alternative endpoints: draining LN weights (WLN) and LNC amounts (CLN). In addition, the ears thickness on different days, the LNC viability and the body weights of all animals were also measured and the differences between the test groups and NCG were compared statistically using ANOVA analysis, the Dunnett-test and the unpaired t-test. Stimulation index (SI), indices of LN weight (IWLN) and LNC amount (ICLN) were also calculated and compared statistically. The results demonstrated that: (1) neat olive oil caused an evident impact on the base level of the LLNA response in CBA/CaOlaHsd mice as indicated by a SI of 4.7* (* $P<0.05$), an IWLN of 1.49* and an ICLN of 2.91*; (2) the draining lymphocyte activity in this strain of mice were significantly increased following repeated exposure to the OO-DMSO mixtures - the SIs were 7.5*, 6.0* and 5.1* at 75%, 50% and 25%, respectively; (3) the draining lymphocyte activity was enhanced in mice treated with neat DMSO with a SI of 2.2* and an ICLN of 1.805* but the effect caused by DMSO was not as marked as that caused by olive oil; (4) the same or similar conclusions were obtained when using WLN and CLN as alternative endpoints as that estimated with the radioactive endpoint (dpm/mouse).

1455 PERFORMANCE CHARACTERISTICS OF THE LOCAL LYMPH NODE ASSAY (LLNA) LIMIT DOSE PROCEDURE.

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ICCVAM recommended the LLNA as a valid substitute for guinea pig tests for assessing allergic contact dermatitis in 1999. In 2007, the CPSC requested that NICEATM and ICCVAM evaluate the validation status of the LLNA limit dose approach, a modification proposed by Kimber et al. (Contact Dermatitis 54:181-185, 2006). In the limit dose procedure, only the high dose is tested compared to testing three or more doses in the standard LLNA. This modification reduces the number of mice used per study by 40% or more. Based on their retrospective evaluation of LLNA data for 211 chemicals, the LLNA limit dose approach, compared to the LLNA, had an accuracy of 98.6% (208/211), a false positive rate of 0% (0/42), and a false negative rate of 1.8% (3/169). Based on this publication, the ECVAM Scientific Advisory Committee (ESAC) concluded in April 2007 that the LLNA limit dose approach could be used to further reduce the number of animals used for skin sensitization testing. NICEATM subsequently obtained additional LLNA data on a total of 465 chemicals and formulations that were used to further evaluate the performance characteristics of the LLNA limit dose approach. Compared to the standard LLNA, the LLNA limit dose approach had an accuracy of 98.9% (460/465), a false positive rate of 0% (0/151), and a false negative rate of 1.6% (5/314). Similar to the three false negatives in Kimber et al., the 2 additional false negatives were classified as sensitizers in the standard LLNA based on the low- or middle dose producing an SI≥3, with the highest dose producing an SI<3. This evaluation of an expanded and more diverse group of chemicals supports the proposed use of the LLNA limit dose procedure. ILS staff supported by NIEHS contract N01-ES 35504.

1456 THE CHEMICAL ALLERGEN NICKEL SULFATE TRIGGERS THE PRODUCTION OF THE BIO-ACTIVE FORM OF INTERLEUKIN-12 BY HUMAN DENDRITIC CELLS.

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Dendritic cells (DC) are professional antigen presenting cells playing a major role in the induction of primary immune responses. Nickel is the most prevalent metallic hapten responsible of about 15% of contact dermatitis. We have previously shown that nickel sulphate can induce DC maturation *in vitro* through upregulation of surface markers (CD40, CD86, CD83 and CMH II) and cytokine production (IL-12p40, IL-6). Moreover, we have shown that this maturation depends on MAPK (Mitogen-activated protein kinase) pathways and on the transcription factor NF-κB (Nuclear factor kappa B). In this study we address the question whether NiSO4 by itself or in synergy with other signals can induce the secretion of Interleukin-12p70 (IL-12p70), the bio-active form of IL-12 by human Mo-DCs (Monocytes derived DC). Mo-DC were obtained from human monocytes purified from peripheral blood and cultured in the presence of GM-CSF and IL-4 for 5 days. In Mo-DC, we showed that nickel alone can trigger the production of IL-p12p40 at 24 hours but not the production of IL-12p70. When we treated our cells with both NiSO4 and IFN-γ, we found a production of IL-12p70 showing that a complementary signal associated to nickel is needed. We then analyzed the expression of IL-12p35 mRNA using RT-PCR and found that IL-12p35 mRNA was expressed only in the presence of NiSO4 and IFN-γ. Both signals induced by NiSO4 and IFN-γ activated different signaling pathways that are complementary for IL-12p70 production. We recently showed that IRF-1 (a member of the family of transcription factors IRF (Interferon Regulatory Factors)) was activated by nickel and may contribute to the production of IL-12p70.

1457 THE EFFECTS OF ASCORBATE AND GLUTATHIONE DEFICIENCY ON THE TOXICITY AND IMMUNOGENICITY OF SULFAMETHOXAZOLE NITROSO IN GUINEA PIGS.

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Sulfonamides such as sulfamethoxazole (SMX) have been associated in humans with hypersensitivity reactions, to include fever, skin eruptions, hepatotoxicity, and blood dyscrasias. It has been hypothesized that SMX hypersensitivity is caused by an inability to detoxify a reactive oxidative metabolite, nitroso sulfamethoxazole (SMX-NO), which can haptenize tissues. Antioxidants such as glutathione (GSH) and ascorbic acid (AA) are known to reduce SMX-NO to the less reactive sulfamethoxazole hydroxylamine (SMX-HA) metabolite. We hypothesized that AA and GSH deficiency would enhance the immunogenicity, and possibly toxicity, of SMX-NO. We tested this hypothesis in guinea pigs fed either a normal or AA restricted diet for 2 weeks, followed by buthionine sulfoximine (BSO; 250 mg/kg SC daily, or vehicle) to prevent GSH synthesis, and SMX-NO (1mg/kg IP 4 days per week, or vehicle) for an additional 2 weeks. Guinea pigs did not show any clinical or biochemical evidence of drug hypersensitivity. Hepatic necrosis and elevated plasma alanine aminotransferase levels were observed in the GSH depleted guinea

pigs dosed with either SMX-NO or vehicle; these changes could not be attributed to SMX-NO. Proliferation of splenocytes in response to SMX-NO *in-vitro* was highest in guinea pigs depleted of both AA and GSH, suggesting enhanced immunologic response to SMX-NO with antioxidant deficiency. Interestingly, protein expression and enzyme activities of cytochrome b5 and its reductase, which are involved in the detoxification of SMX-HA to SMX, were decreased with combined AA and GSH deficiency. These results suggest that antioxidant deficiency may increase the risk of an immune response to sulfonamides, as well as impair sulfonamide detoxification.

1458 A STUDY OF AIRBORNE CHRYSOTILE CONCENTRATIONS ASSOCIATED WITH HANDLING, UNPACKING, AND REPACKING BOXES OF AUTOMOBILE CLUTCH DISCS (CIRCA 1950-1980).

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The objective of this study was to assess chrysotile asbestos exposures that could occur during the handling of asbestos-containing manual clutches. 24 original boxes of unused asbestos-containing clutch discs (manufactured prior to the 1980s) were obtained and airborne asbestos levels were measured while the clutches were unpacked, repacked, and stacked on a counter-top. Breathing zone samples on the lapel of a volunteer worker (n=100), area samples at bystander (n=50), remote area (n=25), and ambient (n=9) locations were collected and analyzed by Phase Contrast Microscopy and Transmission Electron Microscopy. Exposure to airborne asbestos was characterized for a variety of parameters including the number of boxes handled, clutch brand, distance from the activity (ie, worker, bystander, remote area), and duration of exposure (ie, short- vs long-term). In addition, fiber distribution by size and morphology was evaluated according to the ISO analytical method for asbestos. Estimated 8-hr TWA asbestos exposures for a worker handling 1 or 2 boxes of clutches over a workday ranged from 0.002 to 0.006 f/cc, while the estimated 8-hr TWA asbestos exposures for a worker continuously stacking unopened boxes of clutches over a workday was 0.013 f/cc. It was observed that: 1) the clutches contained between 20-52% chrysotile asbestos, 2) amphiboles were not detected in any of the clutches, 3) airborne asbestos concentrations increased with the number of boxes unpacked and repacked, 4) fiber size and morphology analysis showed that little more than half (57%) of the airborne OSHA fibers (fibers greater than 5 µm in length) were free (eg, not associated with a cluster or matrix) and less than 30% of the free OSHA fibers were respirable (fiber diameter < 0.7 µm), and 5) all of the measurements were below current and historical OSHA short- and long-term standards. These data can be used to estimate asbestos exposures for garage mechanics or parts handlers who may have worked with asbestos-containing clutches in the past.

1459 PREDICTING THE IMPACTS OF AGGREGATE AND CUMULATIVE EXPOSURES ON HUMAN HEALTH.

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The concern for Aggregate and/or Cumulative (AC) exposures is based on the observation that exposures to a chemical, or chemicals, occur by multiple routes from multiple sources and therefore the total dose an individual receives of a chemical, or chemicals, will be higher than the dose from any single chemical/source. In this project the size of the differences between doses from single chemicals/sources and AC doses were investigated using simulation models of exposures across a hypothetical population. Cumulative exposures were evaluated using a TEQ-based approach. The sizes of AC doses were compared to the sizes of the maximum dose from any one chemical/source (Max dose). This model was used to investigate the impact of the number of sources, relative sizes of chemical/source doses, and interindividual variation in the different chemical/source doses on the differences between the Max and AC doses in the simulated individuals. The results show that AC doses are much larger than Max doses when individuals receive similar-sized doses from multiple sources. Simulations fitted to data on source intensity of 12 high-volume chemicals; however, found that AC doses were similar to Max doses (i.e. AC doses are dominated by doses from a single chemical/source). This occurs both because of the large variation in the intensity of exposure for individual sources observed in many chemicals and the variation in potency across chemicals. The tendency of one chemical/source to dominate individuals' AC exposures decreases with the number of chemicals/sources and increases for higher percentiles of the distribution of interindividual variation in AC dose and when the interindividual variations in chemical/source doses are large. These findings suggest that for many chemicals (and groups of chemicals) independent assessments of doses by chemical/source will largely address risks from chemicals. In addition, these findings can help identify those instances where AC exposures may greatly exceed exposures from any single source and thus warrant a separate AC assessment.

1460 TAPE STRIPPING FOR THE ASSESSMENT OF DERMAL PYRENE EXPOSURE: A VOLUNTEER STUDY.

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Polycyclic aromatic hydrocarbons (PAH) are known to penetrate the skin of workers in numerous occupational settings. We assessed the characteristics of a tape stripping approach to measure the amount of pyrene, a non-carcinogenic PAH, deposited on the skin of 10 healthy volunteers not occupationally exposed to PAH. Ten µL of a 2.5 g/L solution of pyrene in acetone was applied to 10 cm^2 areas of the volar forearms of the subjects. Cover Roll (# 45554-00, BSN Biomedical) was applied to the skin at various time intervals following pyrene application, extracted with acetonitrile and analysed by HPLC using a fluorescence detector. Measurements made at 0.17, 1.5, 3, 6, and 7.5 h revealed a first-order decay (R^2 = 0.99) with a mean half life (range; median) of 3.7 (1.7 – 7.1; 3.3) h (n = 10). The efficiency of a gentle warm water / liquid hand soap washing was also tested on a separate occasion 0.25 and 6 h following application of pyrene. The right arm was washed whereas the left arm served as positive control for each subject and the efficiency of removal calculated as 100% - % of pyrene measured on the washed arm compared to the positive control arm. This simple washing approach removed 83 ± 10% of pyrene measured at 0.25 h post application and 66 ± 17% at t = 6 h (n = 10). Despite the fact that acetone application of this study is very different from occupational exposure to pyrene, the results suggest that tape stripping is a convenient approach to measuring dermal exposure to PAH in the workplace. Pyrene disappearance rate suggests that there should only be minimal amounts remaining on the skin of workers on the morning following exposure. Furthermore, simple washing of the contaminated skin quickly after exposure should markedly contribute to lowering dermal absorption of PAH.
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1461 ASSOCIATIONS BETWEEN ASTHMA EMERGENCY DEPARTMENT VISITS AND DIESEL EMISSIIONS IN TACOMA, WA.

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Tacoma, WA is the second largest city in the Puget Sound region with approximately 201,700 residents. While several studies have been conducted in Seattle to investigate the health effects of air pollution, there are no studies that have looked at the air pollution effects in Tacoma, a port city that is approximately 32 mi SW of Seattle. We were also interested in the effects of the use of diesel generators during an energy crisis during the winter of 2001. From January 24 to June 2, 30 diesel powered generators were used to supply electricity to Tacoma residents. These generators were shut down completely on June 30. Daily asthma emergency department (ED) visit data were available from 6 Tacoma hospitals from Jan 3, 1998 to May 30, 2002. Only emergency visits where the primary discharge diagnosis was asthma were included in the analysis. Air pollution, daily temperature and relative humidity data were obtained from the Puget Sound Clean Air Agency. We found an association between daily PM2.5 and ED visits for asthma at lag days 2 and 3. The effect estimate for lag day 2 was 1.04 (95%CI: 1.01, 1.07) and for lag day 3 was 1.03 (1.0, 1.06). We did not detect a significant association between ED visits for asthma and increased use of diesel generators. These data indicate that air pollution in a medium sized, coastal city may be sufficient to have a public health impact on asthma.

1462 EVALUATION OF KEY INFORMATION FORMING THE BASIS OF EPA'S NEW RECOMMENDED OZONE STANDARD.

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EPA proposed a new NAAQS standard for ozone in the Federal Register on July 11, 2007, reducing the current standard of 0.08 ppm ozone to a concentration within the range of 0.070 to 0.075 ppm, based on findings in chamber/clinical studies and epidemiology studies. To support their proposed ozone standard, EPA conducted both a quantitative risk assessment and a qualitative evaluation of the results in the studies.

We identified those studies considered "key" by EPA, and summarized the information including both the authors' and EPA's conclusions. Further, we evaluated EPA's use of the studies in developing and supporting their proposed standard, and we evaluated the strength of a causal relationship for a number of endpoints. When we plotted hospital admissions, emergency room visits, and total mortality as a function of ozone concentration (average, 98th percentile, and 99th percentile), we found no indication of a traditional dose-response relationship, with effects increasing as dose or concentration increases, as would be expected based on basic toxicological principles. The lack of an apparent dose-response relationship casts doubt on the role that ozone exposure, particularly at concentrations <0.08 ppm, may play in hospital admission rates, emergency room visits, or total mortality. We also noted inconsistent findings and weaknesses in the mortality studies, and a lack of coherence between data for cardiovascular morbidity and mortality where mortality was elevated, but morbidity was not. Further, chamber studies provide little evidence of significant effects below 0.08 ppm. In conclusion, there is a limited scientific foundation to support a reduction in the present standard. One cannot conclude with a high degree of confidence that a reduction in the present standard will clearly provide substantial health benefits.

1463 NEUROLOGICAL SYMPTOMS AND NEUROPATHOLOGICAL ANTIBODIES IN POULTRY WORKERS EXPOSED TO *CAMPYLOBACTER JEJEUNI*.

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The pathogenic bacterium *Campylobacter jejeuni* is a major cause of food-borne illness and also frequently identified as a risk factor for Guillain Barre Syndrome (GBS), the most common cause of acute neuromuscular paralysis in the world. This association has been found in association with dietary and drinking water exposures, and has been explained in terms of molecular mimicry, in which antibodies to *C jejeuni* surface protein cross react with glycolipid proteins in peripheral nerves.Because of the endemic presence of *C jejeuni* in poultry production, we examined risks of peripheral neuropathy in poultry workers as compared to community referents using a clinical questionnaire developed for studies of GBS. We also examined sera for anti-glycolipid antibodies, a biomarker of GBS, and *C jejeuni*-specific antibodies, a biomarker of *C jejeuni* exposure. Workers were 3 times more likely to report stomach cramping symptoms ($p = 0.03$), and more likely to carry higher titers of anti- *C jejeuni* IgG antibodies ($p = 0.002$). They were also significantly more likely to report neurological symptoms consistent with GBS (4-10 times). Anti-glycolipid IgG antibodies were more common among male poultry workers as compared to male referents ($p = 0.07$). These results confirm associations between *C jejeuni* exposure and risks of peripheral neuropathy. Moreover, the findings suggest that agricultural occupational exposures, in addition to diet and drinking water, may be an unrecognized source of increased risk of this important disease. *Research approved by CHR, JHMI, and supported by the Center for a Livable Future (JHSPH), NIOSH, the Military Infectious Disease Research Program, and NIH.*

1464 EVALUATION OF POTENTIAL INHALATION HAZARDS OF PETROLEUM-, SYNTHETIC- AND BIO-FUELS USING GC/MS ANALYSIS OF VAPORS UNDER EQUILIBRIUM CONDITIONS.

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The increasing attention and evolving policies concerning future energy supplies necessitates understanding the composition of alternative fuels to characterize human exposures to these complex chemical mixtures. A comparison of headspace vapor components of several fuel types was carried out using gas chromatography and mass spectrometry. A preliminary inhalation study of aerosolized Jet Propulsion Fuel 8 (JP-8) and a synthetic Fischer-Tropsch (FT) fuel (S-8) revealed important differences in the hydrocarbon composition of the droplets and vapor phases in the chamber atmospheres. Aromatic hydrocarbons found in JP-8 were replaced with methyl branched isoparaffins and lighter hydrocarbons (C8 - C10) in FT fuel. Headspace vapor results showed a bio-fuel blend of biodiesel and a bio-oil derived from wood chips contained toluene and benzene and some components specific to wood such as Alpha pinene, 2-beta pinene, Limonene, beta-Terpinene, Camphene, 2-methyl furan, and 2,5-dimethyl furan. Such components derived from oils and polymers of plant species may lead to novel exposures compared to petroleum based diesel fuels. More research is needed to characterize the potential exposures to hydrocarbon mixtures derived by different manufacturing processes and these fuels should also undergo standardized toxicological evaluations.

1465 NITRATE TOXICITY RISK ASSESSMENT: FROM FIELD SAMPLING TO REMOTE SENSING AND BEYOND.

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Nitrate poisoning is a common and economically important problem in ruminant livestock production. Toxic nitrate accumulation in plants is linked to climatic conditions that lead to plant stress. We hypothesized that satellite-derived Normalized Difference Vegetation Index (NDVI) data can be used to predict plant stress and nitrate accumulation risk. Kansas State University Veterinary Diagnostic Laboratory forage nitrate assay records from zip code 66427 during 2001-2005 were analyzed in comparison to NDVI and precipitation data. Increased frequency of excessive nitrate accumulation events in forage plants followed declining NDVI during the summer growing seasons associated with summer drought. In addition, nitrate accumulation at 12 sample sites at the Konza Prairie Experimental Station were correlated with dry conditions in the 2007 growing season. These results suggest that risk models for nitrate poisoning in livestock can be developed based on remotely sensed environmental data such as NDVI. Such models will be free of spatial and temporal bias, and provide complete coverage of the land surface area at resolutions relevant to individual animal production units.

1466 EXAMINATION OF THE CURRENT GEOGRAPHIC RELATIONSHIP BETWEEN BLOOD LEAD LEVELS AND AIR LEAD LEVELS IN THE UNITED STATES.

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Sponsor: B. Beck.

We examined the empirical relationship between blood lead levels, lead emissions, and air lead concentrations in the U.S., in light of EPA's recent review and likely revision of the National Ambient Air Quality Standard (NAAQS) for Lead, currently set at 1.5 μg/m3. We developed maps of air lead levels, lead emissions data, and blood lead levels for the U.S. Data for the maximum quarterly average air lead levels by county for 2001 to 2006 were drawn from EPA's Air Quality System (AQS). These data show that air lead levels decreased in many locations over this time period. Lead emissions data for all facilities with lead emissions greater than 2 tons/year for 1999 and 2002 were drawn from EPA's National Emissions Inventory (NEI). These data show that point source lead emissions decreased substantially over this time frame. Blood lead surveillance data for 2005, including screening rates and case incidence rates of levels above 10 μg/dL, were drawn from the Centers for Disease Control and Prevention (CDC) Lead Poisoning Prevention Branch, by county for 34 states. We did not find a geographic correlation between the locations of elevated blood lead levels and elevated air lead levels or distance to the nearest lead-emitting facility. State blood lead screening programs were found to vary substantially in both screening rates and targeted populations, contributing to uncertainties and limitations of this analysis. There is also a paucity of air lead monitoring data near many of the facilities with lead emissions greater than 2 tons/year. Using these three sets of publicly available data, it is difficult to draw conclusions about the magnitude of the relationship between air lead levels and blood lead levels. This suggests that either the current relationship between air lead levels and blood lead levels is very weak, or that existing air lead and blood lead data are too limited to be able to determine the relationship at this time.

1467 EFFECTS OF DEMOGRAPHIC, ENVIRONMENTAL, AND POLYMORPHIC FACTORS ON EMERGENCY DEPARTMENT VISITATION IN ASTHMATIC CHILDREN.

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The purpose of this study was to find a prediction model for determining emergency department (ED) visitation for asthmatic children >18 years of age. With self reported demographics, medical history, symptomology, and polymorphic status of four detoxification enzymes as predictor inputs, multivariate binary logistic regression (BLR) and artificial neural network (ANN) modeling with bootstrapping were used to predict visits to the ED. It was hypothesized that the genetic polymorphisms and environmental exposures, such as environmental tobacco smoke (ETS),

would have an inter-related impact on determining ED visitation. 120 subjects were enrolled in the study. Predictor variables were self-reported by the subject and their parent(s)/guardian(s) via questionnaire. Polymorphic status of the phase I/II detoxification enzymes NQO1, GST M1, P1, and T1were determined using PCR-RFLP on buccal cell samples. In the end, the ANN models found no significant linkage interactions between predictor variables within the model and did not outperform the BLR models. Therefore, the final model was a BLR model with only two of the predictor variables being significant enough for inclusion, childhood age and reporting restricted physical activity (OR 0.787 and 3.099 respectively). The final model had a Naglekerke's R-squared value of 0.258. The percentage of accurately predicting the correct response to ED visitation was 69.4%, while the reference (no ED visitation) was 70.4%. The c-indexes for the final model with 100 bootstrap datasets with resampling were 0.755 for the full model and 0.762 for the bootstrapped model, leaving the corrected model c-index at 0.744. In conclusion, genetic polymorphisms of detoxification enzymes in conjunction with environmental exposures and medical history did not show significant inter-relationships with each other in determining ED visitation. Childhood age and reports of restricted physical activity were the only significant prediction variables at the 0.05 level.

1468 DETAILED ASBESTOS FIBER SIZE AND MORPHOLOGY ANALYSES OF AUTOMOBILE CLUTCH DISCS, BRAKE PADS, AND BRAKE SHOES.

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It has been understood since the 1970's that the disease-producing potential of an inhaled fiber is governed in part by the fiber dimension. Fiber widths of > 0.7 μm are rarely found in the lung and recent government expert panel evaluations have concluded that asbestos fibers < 5 μm in length (ATSDR) or even up to 10-20 μm in length (EPA) probably do not contribute to disease. The objective of this study was to analyze the size and morphology of asbestos fibers in air and bulk samples collected from simulation studies entailing the handling, unpacking, and repacking of 121 asbestos-containing brake pads and brake shoes and 24 boxes of asbestos-containing clutch discs. Samples were analyzed by Phase Contrast Microscopy (PCM), Transmission Electron Microscopy (TEM), and the ISO analytical method (fiber size and morphology analysis) for asbestos. It was observed that: 1) the brakes and clutches contained 3-60% chrysotile asbestos, 2) there were no detectable amphibole fibers (polarized light microscopy analysis with a limit of detection of 1%) in any of the brakes or clutches, 2) ISO analyses of air samples indicated that little more than half of the airborne OSHA fibers (fibers greater than 5 μm in length) were free (e.g., not associated with a cluster or matrix) and approximately one-third of the free OSHA fibers were respirable (fiber diameter < 0.7 μm) when the brakes and clutches were handled. The results of this study indicate that handling unused asbestos-containing brakes and clutches results in low airborne concentrations of respirable asbestiform fibers, all of which were chrysotile. The data also indicate that NIOSH measurements (PCM) of airborne asbestos associated with unused friction products would significantly over-estimate actual levels of respirable OSHA fibers, representing an upper bound worst-case exposure estimate. These data are consistent and supportive of industrial hygiene surveys and epidemiologic studies that have reported automotive mechanics are not at an increased risk of asbestos-related disease.

1469 ASSOCIATION OF THE CYP1A1 G184C POLYMORPHISM WITH LUNG CANCER RISK.

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G184C and G134A polymorphisms of CYP1A1 identified in previous studies are associated with the substitutions of Ala62Pro and Gly45Asp, respectively. We tested the effect of these polymorphisms on the risk of acquiring lung cancer in a matched (gender and age) case-control study of 80 cases and 240 controls of Koreans. Subjects were genotyped with a PCR-sequencing assay, and odds ratios were analyzed in a conditional logistic regression model. The odds ratio of acquiring lung cancer in G184C-bearing individuals was 3.37 (95% confidence interval, 0.89-12.73; P = 0.07), whereas it was only 1.23 (95% confidence interval, 0.68-12.73; P = 0.49) in G134A-bearing individuals. In addition, the odds ratio for G184C-bearing individuals who had smoked was 24.72 (95% confidence interval, 4.48-136.31; P < 0.01), whereas it was 6.65 (95% confidence interval, 2.72-16.28; P < 0.01) for non-G184C-bearing individuals who had smoked. These findings suggest that the G184C polymorphism is associated with an increased risk of acquiring lung cancer, especially for those who have smoked.

1470 CASE STUDIES OF NON-PAINT SOURCES OF LEAD EXPOSURES IN THE UNITED STATES.

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Lead-based paint is often cited as the primary source of children's lead exposures, but exposures to "non-paint" lead sources may now be the cause of the most severe cases of adult and childhood lead poisonings, as well as a major cause of lead poisonings in certain communities. The predominant non-paint sources originate in developing countries and include products such as traditional remedies, earthen ware, foods and spices. In addition, many immigrants with high body burdens of lead from exposures in their native countries settle in the US. US-born children of immigrant mothers with high lead burdens are exposed to maternal lead stores in utero and via post natal breast milk feeding.
The nature and national scope of the "non-paint" lead poisoning problem will be described along with documented case studies of lead poisonings attributed to these sources. A brief historical review of the medical literature on non-paint lead sources as well as examples of traditional customs that predispose certain immigrant groups to non-paint lead exposures will be presented. Guidance will be provided for field recognition of ethnic-specific lead hazards. The importance of effective caregiver interview techniques and cultural sensitivity will also be addressed

1471 OCCUPATIONAL EXPOSURE TO BENZENE THE EXXONMOBIL REFINERY IN JOLIET, ILLINOIS (1977-2006).

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As part of an ongoing assessment of ExxonMobil refineries in the United States, we evaluated the historical workplace concentrations of airborne benzene for the refinery in Joliet, Illinois from 1977 to 2006. The results of 2,291 industrial hygiene air samples (both personal and area) were evaluated in this analysis; 1,167 were considered long-term (≥ 180 minutes) personal samples, and 491 were considered short-term (< 180 minutes) personal samples, while the remainder did not fit into either category. Overall, the results indicate that long-term personal exposures were low and generally below the contemporaneous occupational exposure limits (OEL) over the past 30 years. The typical average daily airborne concentrations for each job category were similar across different exposure zones within the refinery. The arithmetic mean, median, and 95th percentile for all workers on the site were approximately 0.125, 0.016 and 0.494 ppm, respectively. In general, short-term airborne concentrations were well below the contemporaneous short-term OELs. The arithmetic mean, median, and 95th percentile for short-term samples were 0.690, 0.046, and 2.889 ppm, respectively. The highest air concentrations of benzene were associated with equipment preparation tasks. Short-term sampling results did not display job-dependence; that is, individuals performing the same task were subject to air concentrations of benzene that were similar, regardless of job category. This study provides a job and task-focused analysis of occupational exposure to benzene during refinery operations, and it should be useful for estimating exposures to benzene for refineries over the past 30 years.

1472 CANCER MORTALITY IN CHINESE POPULATIONS SURROUNDING AN ALLOY PLANT WITH CHROMIUM SMELTING OPERATIONS (1960-1978).

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An ecological cancer mortality study (observation period 1970-1978) examined Chinese populations exposed to groundwater hexavalent chromium (Cr(VI)) from an alloy plant with chromium smelting operations since about 1960. A 1987 report indicated that there was an elevation in all-cancer mortality (when averaged across 5 agricultural villages) with elevated groundwater Cr(VI) compared to the Province average rate, but it was later clarified that no dose-response relationship was observed between cancer rates and magnitude of exposure. The current study compares the cancer rates for these same 5 exposed villages to those of 4 nearby communities which had no Cr(VI) in groundwater based on their location upgradient from the alloy plant. The use of a local comparison group is expected to improve similarity among unmeasured covariates. The average lung-, stomach-, and all-cancer mortality rates for the 3 agricultural villages which had no Cr(VI) in groundwater were not statistically different from those of the 5 agricultural villages with Cr(VI) in groundwater (village average concentrations in 1965 ranged from 0.004 to 2.6 ppm and were generally of similar magnitude through 1979). The industrial

town in which the Cr(VI) source was located had different demographics and a different pattern of stomach and lung cancers compared to the adjacent agricultural villages. The results of other local investigations on cancer mortality and genotoxicity in the exposed populations are presented. The overall findings do not indicate a coherent pattern of association of lung, stomach, or all-cancer mortality with exposure to Cr(VI)-contaminated groundwater. Observed differences in cancer rates are consistent with the suspected influence of diet, smoking, and other recognized risk factors for the site-specific cancers studied.

1473 INHIBIN B IS A POTENTIAL MARKER OF TESTICULAR DAMAGE PRODUCED BY OCCUPATIONAL EXPOSURE TO ORGANOPHOSPHATE PESTICIDES.

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Organophosphate pesticides (OPs) are considered endocrine disruptors because of their capacity to block or activate hormone receptors and/or altering hormone metabolism and consequently alter male reproductive function. Serum inhibin B is a marker of spermatogenesis and testicular function. Our aim was to evaluate the association between OP exposure, as assessed by the urinary excretion of dialkylphosphates (DAPs), and reproductive hormone profile in male greenhouse workers of Morelos, Mexico. We interviewed and collected blood and urine samples from 104 male flower industry workers. Urinary levels of DAPs (dmp, dmtp, dmdtp dep, detp, dedtp) were measured by gas-liquid chromatography. Serum samples were analyzed for FSH, LH, prolactin, testosterone and Inhibin B by ELISA. The associations between urinary DAPs and serum hormone levels were evaluated by multiple linear regression models. We found that dmp levels were positively associated with testosterone serum levels (β= 0.00009, p=0.005) and negatively with inhibin B (β= -0.00057, p=0.02); dep levels were positively associated with LH (β= 0.00367, p=0.003), prolactin (β= 0.00176, p=0.004) and testosterone (β= 0.00161, p=0.03) and negatively with inhibin B (β= -0.00619, p=0.02). The effects of OP occurred at both levels of the pituitary-gonadal axis but were more pronounced at the gonadal level. The association between urinary DEP and hormone serum levels suggested that chlorpyrifos and oxydemethon played an important role in the effects observed. Inhibin B serum levels were negatively associated with the levels of DMP, DEP and total DAPs, suggesting its potential usefulness as a marker of OP male reproductive effects. This project was partially funded by The Mexican Council for Science and Technology (Salud 2002-C01-7574).

1474 COMPARING GENE EXPRESSION PATTERNS IN BLOOD AND LUNG TISSUE OF IMMUNOLOGICALLY-CHALLENGED RATS EXPOSED TO CONCENTRATED AIRBORNE PARTICULATES.

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Children residing in urbanized areas suffer disproportionately higher asthma-related morbidity and mortality, possibly due to higher levels of environmental asthma triggers such as airborne particulate matter. As part of a larger study of gene-environment interactions conferring differential asthma susceptibility in Detroit children, we explored gene expression patterns in blood and lung tissue from sensitized rats exposed to concentrated airborne particulates (CAPs) [PM 2.5]. Brown Norway rats were sensitized with ovalbumin, then immunologically challenged with either saline or ovalbumin before chamber exposure to CAPs. To measure gene expression differences between saline (control) and ovalbumin (challenged) animals in the presence of CAPs, both lung and blood RNA was isolated and hybridized to Affymetrix rat whole genome chips. A structured permutation approach was used to highlight knowledge-based gene annotative categories exhibiting unexpectedly high ($p < 0.05$) numbers of differentially expressed genes. These GO and KEGG categories included "Cell communication," "Metabolism of xenobiotics," and several immunological signaling categories. Because gene expression is tissue-specific, we did not observe a high concordance between expression changes of particular genes in blood versus lung. However, our approach does suggest that while blood and lung respond to environmental challenges through a unique set of genes, these differentially expressed genes can be grouped into common functional collections. These data will help guide the analysis of pathways and functional gene categories relevant to asthma. (This work was reviewed by EPA and approved for publication but does not necessarily reflect official Agency policy)

1475 HIGHER POLYCHLORINATED BIPHENYL (PCB) LEVELS AND RATIOS IN SKIN LIPIDS RELATIVE TO ADJACENT SUBCUTANEOUS ADIPOSE LIPIDS.

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Five skin and adjacent subcutaneous adipose tissue samples were obtained from human cadavers and cosmetic surgery patients. These ten skin and adipose samples were analyzed for lipid content and PCB congener concentrations. The mean ± SEM lipid-adjusted concentration of total PCBs in skin from cosmetic surgery samples was 1740 ± 499 ppb, some 25-fold higher than in adjacent adipose tissue (124 ± 34.4 ppb). In cadaver tissues, total PCB skin levels were ~70-fold higher (40000 ± 18500 ppb) than adipose levels (609 ± 58.8 ppb). The more toxic congeners with 4 to 6 chlorines were found in similar percentages of the total PCB measurements in skin and adipose tissues (i.e., 36 to 44% for cadavers; 42 to 44% for surgical samples), but like total PCB levels skin concentrations on average remained more than an order of magnitude higher than those in adjacent adipose tissue. As expected, congener distributions appear to change with age (age 70 to 93 for cadavers; age < 40 expected for surgical samples), with the more heavily chlorinated congeners increasing in tissue concentration with age. Both skin and adipose samples showed significantly different proportions of trichlorobiphenyls (~two-fold lower in cadavers) and nonachlorobiphenyls (1.7- to 3.7-fold higher in cadavers) consistent with the slower elimination of more heavily chlorinated congeners. These data indicate PCBs accumulate to a significantly greater extent in the lipids of human skin than in adjacent subcutaneous adipose tissues (i.e., 1 and 2 orders of magnitude difference), and suggests a role for human skin as a major sink or storage depot that is not in equilibrium with adjacent adipose tissues.

1476 PILOT STUDY OF VAPOR REACTION PRODUCTS FROM MIXING DIACETYL AND CHLORINE BLEACH.

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Artificial butter flavoring containing diacetyl has been associated with fixed obstructive lung disease in certain worker populations. It is an oily substance that is cleaned up with hot water, detergents and sanitizing agents including chlorine bleach. Diacetyl was screened for volatile and semivolatile organic reaction products off-gassing from well mixed, heated solutions of bleach alone (6% hypochlorite, 500 ml), diacetyl alone (500 ml), and diacetyl + bleach (100 ml + 900 ml) for up to 35 min in a 4 L test chamber with constant air flow of 1 L/min (18-20 air changes per hour). Vapors were analyzed by EPA TO-13, -14, -15, and -17. Mixing diacetyl with bleach led to an exothermic reaction (liquid temp. 70°C) that immediately generated an irritating yellow vapor. A grab sample taken at 2 minutes after mixing yielded 28,000 mg/m^3 total hydrocarbons including 22,000 mg/m^3 diacetyl and >2,500 mg/m^3 chlorinated volatile organics (CVOCs): chloroform (1,300 mg/m^3), chloromethane (1,100 mg/m^3), dichloromethane (100 mg/m^3), carbon tetrachloride (11 mg/m^3), chloroethane (6.1 mg/m^3), allyl chloride (3.7 mg/m^3) and chloroacetic acid (19 mg/m^3). Similar vapor sampling of heated diacetyl produced 22,000 mg/m^3 total hydrocarbons including 20,000 mg/m^3 diacetyl and <0.6 mg/m^3 CVOCs; heated bleach produced 1.1 mg/m^3 CVOCs. Two chlorinated ketones with structural similarity to phosgene (1,1-dichloro-2-propanone at 0.04 mg/m^3; and 1,1,1-trichloro-2-propanone at 0.08 mg/m^3) and bis(2-chloroethyl)ether (at 0.2 mg/m^3) were found at lower concentrations. These findings suggest the possibility that diacetyl/bleach reaction byproduct exposures may occur during clean-up of diacetyl in the workplace. Though intermittent, such cleaning-related exposures should be more thoroughly evaluated and considered in the etiology of fixed obstructive lung disease associated with diacetyl and other flavoring agents.

1477 INDOOR ENVIRONMENT, ASTHMA, ALLERGIES, SICK BUILDING SYNDROME (SBS) IN THE SWEDISH POPULATION- A LONGITUDINAL COHORT STUDY FROM 1989-1997.

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Concern about possible health effects of indoor air pollution is increasing, both with respect to asthma, allergies, and non-specific symptoms from eyes, upper airways and facial skin. Such non-specific symptoms are common in the general population and even more common in buildings with indoor air problems. Most of these studies have dealt with symptoms among office workers, and there are only

few studies on SBS in relation to domestic exposures. Factors, such as wall-to-wall carpeting, type of ventilation system, high room temperature, low outdoor air supply flow rate, and low air humidity to influence the prevalence of SBS-symptoms. Building dampness is a common indoor exposure, shown to be related to an increased prevalence of both asthmatic symptoms and SBS.
Changes of asthma, allergies, SBS and indoor exposures in the dwellings were studied in an 8-year follow-up study of a random sample of the population (n=466) region in Sweden, initially aged 20-65 y. In total, 348 persons (75%) answered the postal follow-up questionnaire. Some improvements of the home environment were observed. Water-leakage during the last year had decreased from 11.2% to 4.8% (p=0.005), visible indoor mould had decreased from 4.7% to 1.6% (p=0.02), and any sign of building dampness had decreased from 16.1% to 9.5% (p=0.004). Dwellings with mechanical ventilation either in living rooms or bedrooms increased from 11.8% to 16.4% (p=0.014).The prevalence of hay fever (14%) and any types of allergy (25%) was unchanged. A few SBS-symptoms had decreased, e.g nasal catarrh (15% to 8%) (p=0.003), sensation of getting a cold (25% to 15%) (p=0.002). Moreover, the prevalence of current smoking had decreased from 30% to 19% (p<0.001). In conclusion, the number of damp buildings has decreased in Sweden, probably due to better maintenance and other improvements. Moreover, tobacco smoking and some respiratory SBS-symptom decreased during the follow-up period, suggesting improved health conditions.

1478 URINARY 1-HYDROXYPYRENE: A BIOMARKER OF POLYCYCLIC AROMATIC HYDROCARBON EXPOSURE IN A GHANAIAN POPULATION.

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous contaminants in the environment produced by incomplete combustion of organic materials. Several PAHs have been characterized as potent carcinogens. We suspected that a population in sub-Saharan Africa could be exposed to PAHs from a variety of non-occupational sources. Additionally, this population is considered at high risk for aflatoxicosis due to ingestion of aflatoxin (AF) B_1, another potent carcinogen. A randomized, double-blind, placebo controlled phase IIa clinical trial was conducted in 177 Ghanaian volunteers who received NovaSil (NS) clay capsules either as high dose (3.0 g/day), low dose (1.5 g/day), or placebo for a period of 3 mo. NS has been found to prevent aflatoxicosis in multiple animal species. The main objective of this study was to utilize the urinary metabolite 1-hydroxypyrene (1-OHP) as a biomarker of overall PAH exposure. An additional aim was to investigate potential associations of AF exposure with PAH exposure. 1-OHP was measured using HPLC-fluorescence methods. Out of the total urines run, 98.9% had detectable levels of 1-OHP. Urine samples collected at baseline and after 3 mo. of the intervention did not show a significant difference in 1-OHP levels between the placebo and low dose group or between the placebo and high dose group. There was no linear correlation between 1-OHP and AF-albumin adduct levels. These results suggest that this population is exposed to PAHs and that intervention with NS has no effect on 1-OHP levels. (Supported by USAID LAG-G-00-96-90013-00).

1479 ENVIRONMENTAL HEALTH & COMMUNITY REVIEW BOARD (EHCRB): SUPPLEMENTING THE TRADITIONAL IRB.

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Community based research is essential to assess the impact of hazardous chemical exposures. Approval of these research project is often challenging for the traditional Institutional Review Board (IRB),which typically focuses on protecting the rights and ensuring the safety of the individuals involved in medical based clinical trials. Community based research often involves additional ethical, legal, and social considerations beyond those of the specific individuals involved in the study. This presentation examines the concept of community based participatory research and the shortcomings of the traditional IRBs in dealing with ethical issues associated with broader community concerns such as implications for family members, neighborhood groups, or local businesses. The rationale and benefits for expanding the roles and responsibilities of review boards related to community based issues is examined. A proposal is made to develop an Environmental Health and Community Review Boards (EHCRB) that includes the fundamental responsibilities and ethical concept of the traditional review boards with an expanded ethical construct of dignity, veracity, sustainability, and justice with an added emphasis on community. The traditional ethical frame work of the IRB is supplemented with additional focuses of dignity, veracity and sustainability. Only by acknowledging the needs and working with the community can we ensure ethically based and socially responsible research. An EHCRB will allow researchers and community members to more fully address their mutual interest in conducting scientific, ethical, and socially responsible research. This presentation will critically examine the advantages and disadvantages of establishing an EHCRB.

1480 ESTIMATION OF TOTAL DIETARY INTAKE OF PERCHLORATE AND IMPACTS ON SETTING A MAXIMUM CONTAMINANT LEVEL FOR DRINKING WATER.

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Perchlorate (ClO_4^-) is an environmental contaminant often associated with the manufacture and storage of fuels for rockets/missiles. Perchlorate has been identified in drinking water supplies throughout the US, potentially exposing millions of people. Concern has been raised regarding the uncertainties in conducting human health risk assessments for perchlorate, including the contribution of diet to the daily perchlorate dose. Accordingly, we have completed an exposure assessment for perchlorate in foods using exploratory survey data collected by the US FDA. Three separate daily doses were calculated by using the mean (low value), 95% upper confidence limit of the mean (middle value), and 90th-percentile (high value) of perchlorate concentrations in food items. Our results showed that an individual consuming a daily diet of 2,000 calories ingests 9.8, 14.4, or 18.9 micrograms of perchlorate per day, respectively. For a 70 kg adult this translates to daily doses of 0.14, 0.21, or 0.27 μg/kg-day. For this investigation it was necessary to formulate a number of assumptions, including the data collected by the US FDA is nationally representative, and the US population eats according to recommendations in the Dietary Guidelines for Americans 2005. Dietary exposure values have important implications on setting a maximum contaminant level (MCL) for perchlorate as MCLs are derived by incorporating a reference dose (RfD); an estimate of a daily oral exposure, including total intake from both water and food sources, that is likely to be without an appreciable risk of adverse effects over a lifetime. To date, the only organization to derive a legally enforceable MCL for perchlorate is the Massachusetts Department of Environmental Protection whom used a RfD of 0.00007 μg/kg-day. Our results suggested that this value will be exceeded through ingestion of food alone. Because it is easier to cleanup potable water sources than it is to eliminate/reduce perchlorate exposure through the diet, the MCL for perchlorate may need to be set as low as is economically and technically feasible.

1481 ASSESSING POTENTIAL EXPOSURE TO TRANSFERABLE INSECTICIDE RESIDUES FROM THE FUR OF DOGS TREATED WITH A SPOT-ON FLEA CONTROL PRODUCT CONTAINING THE PYRETHROID INSECTICIDE PERMETHRIN.

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Pyrethroid insecticides are heavily used to control nuisance pests in and around the home, including on pets. Consequently, potential human exposure to pyrethroid insecticide residues might be significant and should be evaluated for risk assessment purposes. Seventeen pet dogs were treated with a spot-on flea control product containing permethrin (PER). The insecticide was applied in one continuous motion starting between the shoulder blades and continuing along the backbone to the tail region of each dog. Transferable residues were quantified in cotton gloves used to pet the dogs and in cotton tee shirts worn by children living in the same household as the treated dogs. Urine samples were also obtained from the children and a parent in the same household for metabolite [3-phenoxybenzoic acid (3PBCOOH)] quantification. Samples were obtained on Day 0 (prior to treatment), at 4 hrs., and at 3, 7, 14, and 20 days post-application. This treatment schedule was repeated three consecutive times with the same animal and human subjects. The amount of PER transferred to a glove from the fur at 4 hrs. was 117±33 mg/glove while the amount transferred at 20 days was 0.5±0.2 mg/glove. Tee shirts worn by children at 4 hrs. contained 136±36 μg/g shirt while tee shirts worn on day 20 contained only 3±1 μg/g shirt. A significant increase above pre-treatment levels was observed in children's urinary concentrations of 3PBCOOH at 4 hrs. Additionally, there were significant differences between adults' and children's urinary 3PBCOOH concentrations in both the 4 hr. and 20 day samples. These results indicate that there may be some transfer and absorption of permethrin from this particular spot-on formulation, especially during the first 4 hrs. post-application. However, these results should be interpreted with caution as additional studies (replications) are needed to further quantify and assess potential exposures. (Supported by EPA STAR Grant R828017)

1482 CADMIUM EXPOSURE ASSESSMENT USING TOTAL DIET STUDY AND PROBALISTITC MONTE CARLO SIMULATION IN A CADMIUM-POLLUTED REGION, JAPAN.

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Due to past mining or volcanic activities, there exist cadmium-polluted regions in Japan. Past total diet studies in non-contaminated areas revealed that daily cadmium intake was 45 μg/day in 1977, decreased gradually to less than 30 μg/day since 1985 and sustained at about half of Provisional Tolerable Weekly Intake (PTWI) of 7 μg/kg bw per week. We conducted dietary Cd exposure assessment among 963 farmwomen at a contaminated area in Japan using both market basket method and probabilistic statistical method. Food intakes used were derived from the JMETS study conducted in this district during 2002-2005. Cd contents of food items, including agricultural, marine products, and processed foods, were collected at local food markets and measured their Cd concentrations by ICP-MS. The Monte Carlo simulation was used to estimate distributions of their cadmium intake. When no maximum levels were assigned, the mean, median, and 95 percentile of the estimated cadmium intake were 7.16, 6.29, and 14.20 (μg/kg bw/week), respectively. By market basket method of total diet study, the arithmetic mean, median, and range of cadmium intake were 7.40, 6.64, (1.25-38.98) μg/kg bw/week. Contribution from rice is 51-56% of total Cd intake in this district. The elder age groups showed high intake due to their dietary habits, such as eating more rice and marine products. The probabilistic intake estimate showed slightly lower than estimate by market basket method. However, in this Cd-polluted district, approximately half of study individuals are above PTWI by JECFA. But we could not demonstrate any statistically significant adverse health effects by Cd among the individuals, as previously reported in 44th SOT Meeting (2005) and Environ Res. 97 (1): 83-92, 2005.

1483 DETERMINATION OF A SOUTHERN CALIFORNIA REGIONAL BACKGROUND ARSENIC CONCENTRATION IN SOIL.

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Background metals in soil can prove problematic for risk assessment purposes because metals detected at a site may be comprised of naturally occurring metals, regional anthropogenic contributions or a site-specific release. Arsenic is especially problematic since the risk-based soil concentration is 100-times below typical ambient concentrations. The California Department of Toxic Substances Control (DTSC) established a regional background arsenic concentration in soil that can be used as a screening tool for sites throughout southern California. The term "background" collectively refers to both naturally occurring and anthropogenic ubiquitous concentrations in shallow soil. Data were derived from completed Preliminary Environmental Assessment (PEA) reports for proposed school sites. Site data were combined for each county in southern California, including Los Angeles, Orange, Riverside, San Bernardino and San Diego counties. Los Angeles County had the largest number of sites (19 school sites) and arsenic data points (1097 samples) and will serve as the model for the statistical derivation of background arsenic. A probability plot of the arsenic data clearly demonstrated a classical, lognormal distribution from which outliers were determined using the box plot. The summary statistics for the arsenic data set, excluding the outliers, were calculated and the upper-bound arsenic concentration estimated using both the 95% confidence limit of the 99th quantile of the arsenic data set and a distribution-free, nonparametric analysis. Both statistical methods resulted in an upper-bound arsenic concentration of approximately 12 mg/kg for Los Angeles County. Using the same approach, the upper-bound arsenic concentrations were similar for each of the other southern California counties, resulting in an upper-bound estimate of 12 mg/kg for arsenic in southern California. A similar evaluation is being conducted by DTSC for northern California sites in order to derive arsenic screening levels State-wide.

1484 RESEARCH ON TOXIC CHEMICALS IN THE GREAT LAKES: DEFINING STUDIES TO HELP ENSURE HEALTHY PEOPLE IN EVERY STAGE OF LIFE.

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ATSDR in partnership with the Great Lakes state health departments, academic institutions and federally-recognized tribal governments has been conducting epidemiological studies in the U.S .Great Lakes (GL) region to determine exposure to persistent toxic substances (PTSs) which include PCBs, dioxins, methylmercury, pesticides and others via consumption of contaminated GL fish, and to assess the potential for adverse health outcomes in vulnerable populations in the Great Lakes states.

Overall results of these indicated that for the studied populations that: 1) mothers who had serum PCB levels of 4.7 ppb were less likely to have a male child than mothers with the lowest serum level of 0.7 ppb, 2) a significant decline in birth weight of

approximately 500grams in newborns of mothers who had PCB serum levels > 25 ppb,

3) mothers who consumed two or more fish meals per month the risk of a male child having a birth defect was significantly elevated, 4) women who delivered before 35 weeks gestation were more likely to have mercury levels at or above the 90th percentile level, and 5) in women, but not in men, higher PCB serum levels were associated with increased incidence of diabetes. These research findings are of public health concern. The populations identified are at risk because of their elevated exposures and/or their intrinsic sensitivity to PTSs. ATSDR and its partners has developed prudent public health interventions and risk communication tools to interdict future exposure to toxic chemicals in these vulnerable populations. The health data will be discussed and intervention strategies will be described.

1485 AN *IN-VITRO* ASSESSMENT OF A NANOSCALE, REDOX-SENSITIVE ANTIOXIDANT DELIVERY SYSTEM'S IMPACT ON OXIDATIVE DAMAGE.

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A nanoscale antioxidant delivery system (ADS) was synthesized to target and ameliorate cellular oxidative damage resulting from the production of reactive oxygen species (ROS). High levels of ROS are associated with many disease states and conditions including airway inflammatory responses such as asthma and exposure to reactive airborne nanoparticles. This delivery system incorporates folic acid conjugated to poly(ethylene glycol), for receptor targeting through receptor-mediated uptake, and disulfide linked glutathione (GSH) for redox-triggered antioxidant release. Toxicity of the synthesized nanoparticles was assessed using a Promega (Madison, Wi) CellTiter-Glo viability assay. The uptake of the ADS by Lipopolysaccharide (LPS) stimulated RAW 264.7 cells (murine macrophage) was assessed using both flow cytometry and fluorescence microscopy. Oxidative status was assessed by measuring oxidized dichlorofluoroscein-diacetate (DCF-DA) and dihydroethidium (DHE) as indicators of H_2O_2 and O_2●$^-$ concentration. F_2-Isoprostanes, biomarkers for lipid peroxidation, were also quantified using negative-ion GC-MS. RAW 264.7 cells treated with the GSH containing delivery system showed improved viability over both control cells (37.5%) and cells treated with a delivery system which did not contain GSH (40.7%). Both H_2O_2 and O_2●$^-$ levels were reduced indicating decreased levels of intracellular ROS. Cells treated with the GSH containing antioxidant system also resulted in a 67.8% decrease (from 732 to 235 ng/mg protein) in F_2-Isoprostane concentrations. These results demonstrate the efficacy of targeted delivery of GSH on intracellular ROS concentrations in RAW 264.7 cells. This research also suggests that the conjugation of GSH and other antioxidants on the surface of engineered nanoparticles may reduce cellular oxidative damage and overall cytotoxicity.

1486 COMPARISON OF THE BIOLOGICAL ACTIVITY BETWEEN ULTRAFINE AND FINE TIO2 PARTICLES IN RAW 264.7 CELLS ASSOCIATED WITH OXIDATIVE STRESS.

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Ultrafine or fine TiO2 particles are widely used in the production of white pigments, sunscreens and cleanup techniques. However, currently the knowledge is deficient of cellular response to these particles. The study evaluated and compared the biological activity of ultrafine and fine TiO2 particles in RAW 264.7 macrophages according to an oxidative stress paradigm. We found that in vitro exposure of macrophages with ultrafine or fine TiO2 in the range of 0.5-100 μg/ml did not significantly alter cell viability. Ultrafine TiO2 enhanced intracellular generation of reactive oxygen species (ROS) to a greater extent than fine TiO2 at each exposure dose. Ultrafine TiO2 induced ERK1/2 activation in a dose-dependent manner,

while the fine-TiO2-induced changes were minimal. Phosphorylation of ERK1/2 occurred following 10 min exposure to higher doses of ultrafine TiO2 (above 25 μg/ml). Similarly, ultrafine TiO2 exposure significantly enhanced TNFα and MIP-2 secretion in a dose-dependent manner and its potency was higher than fine TiO2. These findings suggest that at relatively low doses of the particles, ultrafine TiO2 has greater biological activity associated with oxidative stress, such as ROS generation, ERK 1/2 activation, and proinflammatory mediator secretion, in RAW 264.7 macrophages than fine TiO2.

1487 EVALUATION OF *IN VITRO* CELL CULTURE SYSTEMS TO ACCURATELY PREDICT THE PULMONARY TOXICITY OF INHALED OR INSTILLED FINE OR NANO ZINC OXIDE PARTICLES.

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Previous studies have reported little correlation between the relative toxicity of particle-types when comparing lung toxicity rankings following in vivo exposures compared to in vitro, cell culture exposures. This study was designed to assess the capacity of in vitro screening assays to predict in vivo pulmonary toxicity of fine (111 nm, 9.6 m2/g) or nanoscale zinc oxide (90 nm, 12 m2/g) particle-types in rats. Rats were exposed by intratracheal instillation to 1 or 5 mg/kg of nanoscale or fine size zinc oxide particle-types. For the inhalation studies, rats were exposed to aerosols of 25 or 50 mg/m3 for 1 or 3 hours. For both types of in vivo studies, lung inflammation, cytotoxicity, cell proliferative and histopathological endpoints were assessed at several time points postexposure.
For the in vitro component of the study, three different culture conditions were utilized. Cultures of 1) rat lung epithelial cells, 2) primary alveolar macrophages, as well as 3) alveolar macrophage – L2 lung epithelial cell co-cultures were incubated with fine or nano ZnO, and the culture fluids were evaluated for cytotoxicity endpoints (LDH, MTT) as well as inflammatory cytokines (MIP-2, TNF-α, and Interleukin-6) at different time periods – 1 hr, 4 hrs, 24 hrs or 48 hrs.
In vivo exposures to instilled or inhaled fine or nanoscale zinc oxide produced a "metal fume fever" type of response, with extended but transient lung inflammatory or cytotoxic responses which were resolved after a few days postexposure. Alternatively, in vitro exposures to fine or nanoscale ZnO produced cytotoxic responses at 4 or 24 hrs but not 1 or 48 hrs incubation in L2 cells and co-cultures, but not in macrophages. Cytokine generation (MIP-2, TNF-α, and Interleukin-6) in ZnO-exposed cells was not significantly different from controls at any dose or time period. To summarize, the comparisons of in vivo and in vitro toxicity measurements demonstrated little correlation, particularly when considering many of the variables assessed in this study.

1488 ASSESSING THE PULMONARY IMPACTS OF TWO SELECTED AMORPHOUS SILICA NANOPARTICLE SIZE RANGES IN RATS FOLLOWING INHALATION EXPOSURES.

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Few data have been generated on the pulmonary toxicity of inhaled nanoparticles per se. This research was designed to systematically evaluate the role of nanoparticle size on pulmonary toxicity parameters in rats exposed by inhalation to smaller (30 nm) vs. larger (70 nm) sized silica nanoparticles. The experimental design was divided into four parts: 1) nanoparticle generation, 2)nanoparticle characterization, 3) stability over time, and 4) mammalian pulmonary hazard effects. To initiate this experimentation, aerosol nanoparticle test materials of untreated, as-synthesized, amorphous SiO2 in two different nanoparticle size range populations (i.e. number distributions centered at d50 = 30 nm and d50 = 70 nm) were developed. Each of the size populations was synthesized in situ and upstream of the inhalation apparatus. The aerosolized nanoparticle populations were tested for stability over time by measuring chamber temperatures and humidity. Groups of 5 animals were exposed to 1.5 × 10E7 particles/cm3 for either 1 × 5 hour period or for 3 × 5 hour periods over 3 consecutive days to establish a dose-response relationship at each post-exposure time-point (24 hrs, 1 wk, and 1 mo). We also conducted a flow cytometric assessment of induction of micronuclei in peripheral blood samples. During the nanoparticle aerosol exposures to rats, particle characterization data was collected at t=0, 1, 2.5, 4, and 5 hours. Characterization results demonstrated precise size populations with 5% size distributions; chemical composition (SiO2), surface charge (-30 mV), and amorphous structure remained identical over the exposure duration. Subsequent post-exposure pulmonary toxicity results (including BAL fluid inflammatory and cytotoxicity endpoints) demonstrated little difference in response to the two different sized nanoparticle-types when comparing exposures to the two different nanoparticle size populations or to sham-exposed air controls. Genotoxicity and lung tissue analysis studies are on-going.

1489 MECHANISMS OF NANODIAMOND PARTICLE INDUCED IL-8 EXPRESSION IN HUMAN AIRWAY EPITHELIAL CELLS.

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Nanodiamond particles (NDP) prepared by detonation under confined conditions have a number of industrial and analytical applications. Previous *in vitro* studies have reported NDP to be biologically inert with negligible cytotoxicity, implying that they are potentially suitable for biomedical applications such as drug delivery. Separate studies have shown that elemental carbon particles that simulate the carbonaceous core of combustion derived airborne particulate matter can induce inflammatory responses in the lung through the generation of reactive oxygen species. To assess the respiratory effects of exposure to NDP, we examined its effects on IL-8 expression by human airway epithelial cells (HAEC) *in vitro*. Four hour exposures of HAEC to 66 μg/ml NDP (average particle diameter of 5 nm and surface area ~300 m^2/g) resulted in IL-8 mRNA increases up to 100-fold over resting levels and was accompanied by up to 13-fold increases in IL-8 protein levels in the media. Transfections with IL-8 promoter constructs showed that exposure to NDP increases IL-8 transcriptional activity. Separate experiments indicated that HAEC avidly take up NDP and that this uptake can be blocked with cytochalasin D. Furthermore, cytochalasin D also abrogated NDP induced IL-8 mRNA levels. NDP-induced IL-8 expression was blunted by overexpression of catalase, and to a lesser extent SOD, in HAEC. We conclude that NDP induce IL-8 expression via a transcriptional mechanism that requires particle uptake and involves the formation of reactive oxygen species. These data are evidence of an inflammatory response to NDP exposure in human lung cells. THIS ABSTRACT OF A PROPOSED PRESENTATION DOES NOT NECESSARILY REFLECT EPA POLICY.

1490 GOLD NANOPARTICLES INDUCE OXIDATIVE DAMAGE IN LUNG FIBROBLASTS *IN VITRO*.

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Scope: Nanomaterials are found to have many uses and potential applications in the fields of biology and medicine. Gold nanoparticles (AuNPs) in particular are being developed as novel gene and drug delivery agents as well as various imaging systems due to due to the inertness of bulk gold. Recent research findings have brought to light concerns over the safety of nanomaterials and long-term adverse effect of their use. In this study, we exposed AuNPs to lung fibroblast in vitro and studied the adverse effects of the nanoparticles to the cells.
Experimental procedures: MRC-5 human lung fibroblast cell line was exposed to 20 nm AuNPs at three concentration (0.1, 0.5, and 1 nmol particle per liter) for 24, 48, and 72 hours. Cell proliferation and viability were evaluated using trypan blue exclusion. TEM was used to image AuNPs internalized by the fibroblasts. To ascertain if AuNPs induce oxidative DNA damage, we analyzed the quantity of 8 hydroxydeoxyguanosine (8OHdG) which causes mutagenicity through G-C to T-A transversions resulting in 8OHdG-A mispaired bases.
Results: There were no visible alterations in the cell morphology between the treated and control (media only without AuNPs) groups at different time points, however, cell proliferation showed a significant difference in the total number of cells at 72 hours following AuNP treatment. AuNPs taken up by the fibroblasts showed up as dark dense clusters under TEM. They mostly gathered in clusters inside cellular vesicles. In some cases, scattered AuNPs were found in the cytosol. DNA damage analysis showed that 8OHdG/106dG value in 1 nmol particle concentration AuNP treated cells was significantly higher than the control, implying oxidative DNA damage when the cells were exposed to higher concentration of AuNPs.
Conclusions: AuNPs may induce some degree of cytotoxicity in human lung fibroblasts via inhibiting cell proliferation and oxidative damage.

1491 PULMONARY RESPONSE TO INTRATRACHEAL INSTILLATION OF FINE OR ULTRAFINE CARBON BLACK OR TITANIUM DIOXIDE: ROLE OF SURFACE AREA.

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Nanparticles are characterized by having a high surface area per mass. Therefore particle surface area may play an important role in determining the biological activity of nanoparticles. However problems arise because nanoparticles tend to agglomerate into μm sized particles when suspended in PBS.

Our previous studies found that such agglomeration in PBS results in inaccurate dose delivery this leads to misinterpretation of toxicity of the particulate being assessed. We found that acellular BALF is effective in dispersing nanoparticles without masking the particles biological surface activity. After establishing an accurate protocol for dispersing nanosized particles, assessment of the inflammatory and cytotoxic potential of ultrafine and fine carbon black and TiO2 was conducted. Fisher rats were exposed by intratracheal instillation to varying doses of ultrafine and fine carbon black or TiO2. At 1,7, or 42 days post-exposure, inflammatory and cytotoxic potential of each particle type was compared on both an equal mass dosage (mg/rat) as well as an equal surface area dosage (cm2 of particles per cm2 of alveolar epithelium). The findings of the study show that on an equal mass basis the ultrafine particles were significantly more inflammogenic and cytotoxic than the fine sized particles. However, when doses were equalized based on surface area of particles given, the ultrafine particles were only slightly more inflammogenic and cytotoxic when compared to the fine sized particles. It was also noted that at 1 day post-exposure, with a dose equalized to surface area, the carbon black particles and the TiO2 particles exhibited similar inflammogenic potential. Over the post-exposure time course of 42 days pulmonary toxicity of both the ultrafine and fine particles tended to resolve. However at 42 days post-exposure the TiO2 was significantly more potent than the carbon black particles. In conclusion this study suggests that surface area of particles may be a more accurate dose metric for pulmonary toxicity studies than mass of particles.

1492 ENGINEERED TITANIUM DIOXIDE NANOWIRE TOXICITY *IN VITRO* AND *IN VIVO*.

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While the application and benefits of manufacturing nanowires is highly promising, their adverse effects have not been fully investigated. For these studies, TiO_2 nanowires (anatase, diameter=80 nm, length=20-25 μm) were synthesized using sol-gel process directed by a porous anodic aluminum oxide template. We conducted in vitro studies using alveolar macrophages isolated from both C57Bl/6 and Balb/c mice assessing toxicity with 4 hr suspension incubations by trypan blue exclusion and apoptosis using Cell Death ELISA. TiO_2 nanospheres caused no toxicity or apoptosis up to 200 μg/ml. In contrast, TiO_2 nanowires caused significant and marked dose-dependent toxicity and increase in apoptosis. Furthermore, TiO_2 nanowires, but not nanospheres increased alveolar macrophage antigen presenting activity (using ovalbumin and T cells from DO11.10 mice) to similar extents as the potent particle crystalline silica. For in vivo studies we exposed C57Bl/6 mice by pharyngeal aspiration to TiO_2 nanowires (0-80 μg/mouse) and examined lung and brain responses at one day post-exposure. In the lung, exposure to TiO_2 nanowires induced dose-dependent increases in the expression of the inflammatory mediators TNF-α (1.8- to 5-fold), MIP-2 (4- to 33-fold) and CCL2 (7- to 30-fold). In the brain, pulmonary exposure to TiO_2 nanowires induced expression of the endothelial cell adhesion molecule E-selectin in olfactory bulb (4-6 fold), suggestive of altered blood brain barrier (BBB) permeability. Unlike the dose-dependent pulmonary effects, the neural responses were elicited only by higher doses of the nanowires. Whether the BBB changes observed are a consequence of the translocation of these nanoparticles to the brain or a systemic inflammatory response remains to be investigated. Taken together, the data suggest that exposure to TiO_2 nanowires may result in adverse health outcomes. Supported in part by grants ES-015497 and RR-017670 (A. Holian, PI)

1493 COMPARATIVE PULMONARY RESPONSE TO INHALED MULTIWALLED CARBON NANOTUBES, CARBON BLACK, AND ALPHA QUARTZ.

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Baytubes® is a Multiwalled Carbon Nanotubes (BT-CNT) material that consists of large agglomerates. To make inhalation testing possible micronization was necessary. Wistar rats were exposed for 1x6 h by inhalation to aerosolized crystalline quartz(Q), carbon black (Printex® 90, P90; BET: 300 m2/g), and two concentrations of BT-CNT (BET: 250 m2/g), at 248, 229, 11, and 241 mg/m3, respectively. The MMAD was for Q, P90, and BT-CNT low/high 2.3, 2.0, 2.9/2.2 um (GSD 1.8-2.6). Rats (6/serial sacrifice) were examined on postexposure days 7, 28, and 90. Inflammatory endpoints were determined in bronchoalveolar lavage (BAL). The concentration of Cobalt in lungs, lung-associated lymph nodes (LALN), brain, kidneys, testes, and liver were analyzed as a translocation marker for BT-CNT. The H&E and Sirius Red stained lung sections of rats exposed to BT-CNT were examined by histopathology (days 28 and 90). In Q-exposed rats the LALN weights increased time-dependently. By contrast, those of CB and BT-CNT exposed rats declined to the level of the control. BAL-protein, LDH, and –collagen showed a similar trend. The influx of neutrophilic granulocytes (PMNs) was most pronounced in Q-exposed rats. At 11 mg BT-CNT/m3 (day 90), most endpoints in BAL were similar to the control or CB groups. Histopathology revealed an increased cellularity in the bronchioloalveolar region (focal septal thickening and fibrosis) at 241 mg/m3. The distinct time-related exacerbation observed in Q-exposed rats did not occur in BT-CNT or P90-exposed rats. Despite a concentration-dependent increase of Co in lung tissue translocation to extrapulmonary organs did not occur. The clearance of Co during the 3 months postexposure period was 96% and 43% at 11 and 241 mg BT-CNT/m3, respectively. In summary, at the end of the 3-months postexposure period, the time-related exacerbation observed following exposure to Q was not seen in rats exposed to micronized BT-CNT.

1494 COMBINED EXPOSURE TO CARBON NANOTUBES AND BACTERIA ENHANCES PULMONARY INFLAMMATION AND INFECTIVITY.

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Carbon nanotubes (CNT) – with their applications in industry and medicine - may lead to new risks to human health. CNTs induce a robust pulmonary inflammation and oxidative stress in rodents. Realistic exposures to CNT may occur in conjunction with other pathogenic impacts (microbial infections) and trigger enhanced responses. We evaluated interactions between pharyngeal aspiration of single walled CNT (SWCNT) and bacterial pulmonary infection of C57BL/6 mice with Listeria monocytogenes (LM). Mice were first given SWCNT (0, 10 and 40 μg/mouse) and 3 days later exposed to LM (10^3 bacteria/mouse). Combined exposure to SWCNT/LM amplified lung inflammation and fibrosis. Despite this robust inflammatory response, SWCNT pre-exposure significantly decreased the pulmonary clearance of LM measured 3 - 7 days after microbial infection vs PBS/LM treated mice. Failure of SWCNT-exposed mice to clear LM led to a continued elevation in nearly all major chemokines and acute phase cytokines into the later course of infection. In SWCNT/LM exposed mice, BAL PMNs, AMs and lymphocytes, as well as LDH levels, were increased compared to mice exposed to SWCNT or LM alone. Increased levels of collagen deposition were found in the lung of mice exposed to SWCNT/LM vs SWCNT or LM alone. Combined exposure to SWCNT/LM caused persistent changes in breathing rate patterns indicative of detrimental decline in the lung function. In conclusion, enhanced acute inflammation and pulmonary injury with delayed pulmonary bacterial clearance after SWCNT exposure may lead to increased susceptibility to lung infection in exposed populations.

1495 COMPARATIVE PULMONARY TOXICITY STUDY OF 3 DIFFERENT PRIMARY-SIZED TIO2 PARTICLES IN RATS.

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Lung toxicity of 3 different-sized, well-characterized, anatase TiO2 particles was assessed in rats. The average primary size of these particles was approximately 7, 20, and 200 nm, respectively. Groups of male Crl:CD (SD) rats were intratracheally instilled with 5 mg/kg of the TiO2 particles dispersed in disodium phosphate solution. Following the instillations, the bronchoalveolar lavage fluid (BALF) of the rats was examined for inflammatory markers, and the histopathology of the lung, liver, spleen, and cerebrum at post-instillation timepoints of 24 hours, 3 days, 1 week, and 4 weeks was also examined.

In all the groups, toxicological effects were observed only in the lung and not in the liver, spleen, or cerebrum. The pulmonary inflammatory responses were different among the groups. In the 7 nm-TiO2-instilled group, the BALF measurements indicated significant increase in total cell and neutrophil numbers and in LDH and IL-6 concentrations. Further, macrophage infiltration in the alveoli and interstitium, inflammatory-cell infiltration, and hypertrophy of the alveolar epithelium cells were observed in the histopathological evaluations. In the 20 nm-TiO2-instilled group, the inflammatory responses were almost the same as in the 7 nm-TiO2-instilled group. However, no significant increases in the IL-6 concentrations in the BALF, or macrophage infiltration in the interstitium were observed. In the 200 nm-TiO2-instilled group, no significant differences were observed for any of the inflammatory biomarkers in the BALF. Further, in the 200 nm-TiO2-instilled group, the levels of the macrophage infiltration in the alveoli and inflammatory-cell infiltration were much lower than those observed in the 7 nm- TiO2 or 20 nm-TiO2-instilled groups.

All the above-mentioned TiO2 particle types produced only transient pulmonary inflammatory effects, and these changes were recovered at 1 month after the instillations.

1496 COMPARATIVE PULMONARY TOXICITY STUDY OF 3 DIFFERENT DISPERSIONS OF NANO- TIO2 PARTICLES IN RATS.

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In order to evaluate the effects of size of poorly soluble, low-toxicity (PSLT) particles on pulmonary toxicity, not only the effects of the primary sizes of these particles but also the effects of their dispersion need to be examined.
The aim of this study was to assess the lung toxicity of 3 different, well-characterized dispersions of anatase nano-TiO2 particles in rats. The average secondary particle size of these TiO2 particles, which were prepared from the same primary-sized nanoparticles, was approximately 18, 65, and 300 nm. Groups of male Crl:CD (SD) rats were intratracheally instilled with 5 mg/kg of these nano-TiO2 particles dispersed in 0.2–1.3% of disodium phosphate (DSP) solution. DSP solution- and Min-U-Sil quartz-instilled rats served as vehicle and positive controls, respectively. Following the instillations, the bronchoalveolar lavage fluid of the rats was examined for inflammatory markers such as the number of white blood cells, LDH and protein. The histopathology of the lung, liver, kidney, spleen, and cerebrum at post-instillation timepoints of 24 hours, 3 days, 1 week, 4 weeks, and 3 months was also examined.
Mild inflammatory responses in the lung were observed in the 18 nm- and 300 nm-sized TiO2 particle-instilled groups at 24 hours, 3 days, and 1 week after the instillations. In contrast, in the 65 nm-sized TiO2 particle-instilled group, only minimal inflammatory responses were observed in the lung at all the timepoints. At 4 weeks after the instillation, the lung inflammatory responses were comparable among these 3 TiO2 groups and the vehicle control group. Thus, it was concluded that these TiO2 particle types produced only transient pulmonary inflammatory effects. In all the groups, no histopathological changes were observed in the liver, kidney, spleen, or cerebrum.

1497 INHALATION OF CARBON NANOTUBES INDUCES OXIDATIVE STRESS AND CYTOKINE RESPONSE CAUSING RESPIRATORY IMPAIRMENT AND PULMONARY FIBROSIS IN MICE.

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Due to their unique physico-chemical, electrical, mechanical and thermal properties, single walled carbon nanotubes (SWCNT) have numerous applications in electronics, aerospace devices, and computers, chemical, polymer and pharmaceutical industries. Previously, we have reported that pharyngeal exposure of C57BL/6 mice to SWCNT caused dose-dependent formation of granulomatous bronchial interstitial pneumonia, fibrosis, oxidative stress, acute inflammatory/cytokine responses and decrease in pulmonary function. In this study, we investigated whether whole-body inhalation exposure to aerosolized SWCNT (using generation system developed at NIOSH, Baron et al., 2007) caused pulmonary toxicity and early onset of fibrogenic response similar to adverse pulmonary toxicity reported previously. Here, we report that both inhalation and aspiration exposures to unrefined SWCNT (HiPco, CNI, Inc, TX) resulted in an augmentation of biomarkers of inflammation and oxidative stress as evidenced by ESR detection of POBN-spin trapped carbon centered radicals and significant depletion of antioxidants in mouse lungs, recruitment of inflammatory cells and increase in pro-inflammatory cytokines assessed in the bronchoalveolar lavage (BAL) fluid, and early onset of granulomatous lesions and interstitial pulmonary fibrosis. The chain of pathological events in both exposure routes was realized through synergized interactions of early inflammatory response and oxidative stress culminating in the development of multifocal granulomatous pneumonia and robust interstitial fibrosis.

1498 CYTOTOXICITY OF NANOSIZED TIO2 AND MULTI-WALLED CARBON NANOTUBES ON CELL PROLIFERATION *IN VITRO*.

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There is growing concern that as commercial development of nanosized materials develops there is a corresponding need for safety assessment of these materials. Recent studies have demonstrated granuloma formation, fibrosis and cytokine mediated inflammation associated with pulmonary exposure to a variety of nanosized materials. In this study TiO2 and Multiwalled carbon nanotubes (MWCN) were tested in 4 cell lines primarily associated with the lung: A549 human bronchial epithelial cells (hBEC)(ATCC # CCL-185), L2 rat bronchial epithelial cells (rBEC)(ATCC # CCL-149, rat alveolar macrophages(AM)(ATCC# CRL-2192) and CHOK1 cells. To test the relative toxicity of these materials each cell line was grown to confluence in appropriate media and treated for 48 hrs with serial dilutions of TiO2 and MWCN of 0.001 to 30μg/μl. 6 well dishes containing 1x10 5 cells/well were treated. TiO2 and MWCN were suspended in appropriate media and sonicated for 30 minutes prior to treatment. All plates grew for 48 hrs at 37o C and 5% CO2. Wells containing cells with no treatment served as negative controls while H2O2 served as the positive control. The concentration of the materials that suppressed the cell numbers by 50% in comparison to the untreated controls (IC50) was determined and used as an index for cytotoxic activity. Three independent assays were performed with each material in duplicate per concentration. The results were that the IC50s for the MWCN were 0.06, 0.5, 0.4, and 2.0μg/μl for AM, hBEC, rBEC and CHOK1, respectively. For TiO2 the IC50 values were 0.04, 0.3, 0.01 and 0.8 μg/μl for AM, hBEC, rBEC and CHOK1. Confocal microscopy showed extensive uptake of the nanomaterials by AM which may account for their greater sensitivity. Also, rBEC cells were very sensitive to TiO2 suggesting that the rat may not be an ideal model for human toxicity of this material. Funding for this study was provided by the HESI Nanomaterial Safety Committee.

1499 DIRECT FIBROGENIC EFFECTS OF DISPERSED SINGLE WALLED CARBON NANOTUBES ON HUMAN LUNG FIBROBLASTS.

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Nanomaterials, including single walled carbon nanotubes (SWCNT), are being developed for a variety of commercial products. However, toxicity of these new materials has not yet been identified due to limited information regarding the relationship between their unique physicochemical properties and potential toxicity. We reported recently that dispersed SWCNT (DSWCNT) exposure in mice caused a rapid and progressive interstitial lung fibrosis without a persistent inflammation. From these studies, it was hypothesized that DSWCNT, due to their exceptionally small size, can quickly penetrate the alveolar epithelial wall, enter the underlying tissue, and induce a "direct" fibrotic effect by interacting with cells at the site of particle accumulation to induce collagen deposition. The present study investigated the effects of *in vitro* DSWCNT exposure of human lung fibroblasts on proliferation and collagen production using cell multiplication (MTT), and Western blot techniques. DSWCNT were prepared using methods described previously in our laboratory. The results demonstrated that: 1) pulmonary exposure to DSWCNT (10 μg/mouse) did not cause significant lung injury; however, DSWCNT did penetrate into the interstitial lung tissue as analyzed by electron microscopy; 2) DSWCNT stimulated fibroblast cell growth *in vitro* at doses of 0.3-1 μg/ml; 3) DSWCNT also induced collagen production and collagenase (MMP9) activation in the treated fibroblasts. We also observed that the dispersion status or size of the SWCNT is a critical factor in determining fibrogenicity. These findings provide new insights into the mechanisms of SWCNT-induced lung fibrosis and may aid in developing models for risk assessment of these nanoparticles.

1500 PULMONARY RESPONSE TO INHALED NICKEL NANOPARTICLES.

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Nanotechnology is a rapidly growing field which utilizes nanosized materials with unique properties (i.e. biological). Presently, there are concerns about the potential health effects that these materials may cause. For this reason, it is important that research is conducted that addresses specific questions concerning the safety and hazards of these materials. An exploratory study was conducted to assess the pulmonary toxicity of acute exposure to nickel nanoparticles (Ni NPs) (CMD=50nm) in male C57BL/6 mice. Utilizing a whole-body exposure system, male mice inhaled either spark generated Ni NPs (GFG-100 Palas, Germany) or filtered air for 4hrs at the following concentrations: 1000, 150, or 33 μg/m3. Bronchoalveolar lavage fluid was collected 24 hours post-exposure to assess neutrophil infiltration and protein leakage into the lungs. A neutrophil population of 28%, 5%, and 2% was observed for 1000, 150, and 33 μg/m3 Ni NPs exposed mice, respectively. Compared to filtered air exposed mice, only the 1000 and 150 μg/m3 data were statistically significant. No difference in the protein levels was observed at any of the concentrations used. These results illustrate a dose-dependent inflammatory response to acute exposures of Ni NPs. Gene expression analyses using real time reverse transcription-polymerase chain reaction (RT2-PCR) showed increased 1L-6, HO-1,

and TNFa expression in lung tissues collected from Ni NP exposed mice. Ni NPs were characterized using an atomic force microscope, a differential mobility analyzer, and a transmission electron microscope. Additional studies are needed in order to further assess the observed inflammatory responses and to determine subsequent systemic effects.

1501 PULMONARY TOXICITY ASSOCIATED WITH NON-DISPERSED TITANIUM DIOXIDE NANORODS.

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The potential for pulmonary toxicity of nanoparticles after respiratory exposure may depend on particle size, shape, composition, and reactivity. The goal of this study was to characterize lung toxicity and inflammation associated with non-dispersed titanium nanorods (TiO_2), a particle considered to be relatively inert at larger sizes. The reactivity of rutile TiO_2 nanorods (10 x 40 nm) was assessed by electron spin resonance (ESR). Also, ESR was performed on rat alveolar macrophages (AMs) that had been treated for 3 min with TiO_2 nanorods *in vitro* (10 μg, 50 μg, 100 μg, or 1 mg) or a positive control (silica) to measure particle-induced radical production. To assess the pulmonary effects *in vivo*, male Sprague-Dawley rats were intratracheally instilled (IT) with saline or TiO_2 nanorods (10 μg, 100 μg, or 1 mg per rat). On days 1, 3, and 6 post-IT, the left lungs of rats were preserved for EM and histopathological analysis, and bronchoalveolar lavage (BAL) was performed on right lungs to retrieve cells and fluid for analysis. ESR showed a significant dose-dependent presence of hydroxyl radicals in the TiO_2 samples, and a dose-dependent production of hydroxyl radicals in AMs stimulated with the particles when compared to controls; however, the hydroxyl radical signals of the TiO_2 samples were low compared to the positive control. *In vivo*, rats treated with 1 mg of TiO_2 nanorods had significantly elevated levels of lung cytotoxicty, neutrophil influx, cellular oxidant production, and inflammatory cytokines and chemokines (IL-6, MCP-1, and MIP-2) on days 1 and 3. Rats exposed to 100 μg of TiO_2 nanorods also had significant increases in the same indicators of cytotoxicty, inflammation, and oxidative stress when compared to controls on day 1, although to a lesser degree than the 1 mg group. To summarize, the non-dispersed TiO_2 nanorods were found to induce radical formation and oxidant production, and were acutely cytotoxic and inflammatory in the lung. Future studies will assess these responses using dispersed TiO_2 nanorods.

1502 A NINETY-DAY SUBCHRONIC INHALATION TOXICITY STUDY OF SILVER NANOPARTICLES IN SPRAGUE DAWLEY RATS.

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The use of silver nanoparticles due to their antibacterial effect has applied extensively in health, electronic, and home products. Especially inhalation toxicity of silver nanoparticle is foremost urgent information to ensure workers and consumers'health. The inhalation test method was performed according to the OECD test guideline No. 413. The 8-week-old rats were divided into 4 groups (20 rats/group, male and female): fresh-air control, low (0.66×10^6 particle/cc, 49 $\mu g/m^3$), middle (1.43×10^6 particle/cc, 133 $\mu g/m^3$), and high-dose group (2.85×10^6 particle/cc, 515 $\mu g/m^3$). The animals were exposed to silver nanoparticles for 6 hours/day, 5 days/week, for 13 weeks in the inhalation chamber. The male rats did not show significant changes in body weight during the 90-day experiment, but the body weight of female rats showed significant changes in the middle-dose group compared with the high-dose group from 21-days of silver nanoparticle exposure. In the pulmonary function test, male rats exposed to SNPs showed a statistically significant and dose-dependant decreases of tidal volume after 90-day of silver nanoparticle exposure, while female rats showed a significant decrease in the middle-dose group. There were no significant changes in food consumption, the hematology values in both male and female rats. There were no specific symptoms in opthalmological examination and urinalysis in both male and female rats. The blood biochemical values and organ weight did not show dose-dependent changes in both male and female rats. Our results indicated that exposure to Silver nanoparticles concentration near the current ACGIH silver dust TLV (100 $\mu g/m^3$) may have influence on the pulmonary function.

1503 COMPARISON OF HEALTH EFFECTS AND COMPOSITION OF BIOGENIC SECONDARY ORGANIC AEROSOLS FORMED WITH AND WITHOUT SULFUR DIOXIDE.

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We present initial findings from the Secondary Particulate Health Effects Research (SPHERES) program, whose main objective is to define the composition and resulting relative health hazard of secondary organic aerosol (SOA) synthesized under varying reaction conditions. These initial findings will focus on the comparisons among atmospheres generated with an α-pinene hydrocarbon precursor. Reactions were conducted in a 11 m3 continuous flow stir reactor ("irradiation chamber"), which permitted constant production of SOA for the conduct of inhalation exposures in laboratory rodents. We will report on the comparison of results of SOA atmospheres produced with α-pinene:nitrogen oxides (neutral atmosphere) and α-pinene:nitrogen oxides:sulfur dioxide (acidic atmosphere). With both sets of precursors, SOA atmospheres were produced to yield 200 μg/m3 particulate material. Direct comparisons of composition and toxicity were conducted with normalization of atmospheres to either total particulate matter or total organic particulate matter. Exposures were conducted down-stream of honeycomb denuders employed to remove the gas-phase precursors and reaction products. Nose-only exposures were conducted with either Sprague Dawley rats (pulmonary effects) or ApoE mice (pulmonary and cardiovascular effects). Chemical analysis of generated atmospheres confirmed the presence of ambient tracers and organosulfur compounds that have previously been reported in ambient air. SOA had an approximate 100 nm particle size. Neither exposure atmosphere induced an inflammatory response measured by pulmonary cell infiltration; however, the acidic atmosphere upregulated markers of vascular injury and oxidative stress (ET-1, MMP9, and HO-1). Work supported by the Electric Power Research Institute.

1504 TECHNICAL CHALLENGES ASSOCIATED WITH ASSESSING THE *IN VITRO* PULMONARY TOXICITY OF CARBON NANOTUBES.

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Nanotechnology continues to produce a large number of diverse engineered nanomaterials (NMs) with novel physicochemical properties for a variety of applications. Test methods that accurately assess/predict the toxicity of NMs are critically needed and it is unclear whether current test methods are adequate. This study presents the challenges and results associated with assessing the effects which purified single-wall carbon nanotubes (SWCNT), produced by various methods, have on the cell growth/viability of the human airway BEAS 2B cell line. Residual oil fly ash (ROFA) was employed as a positive control in this study. SWCNTs were optimally dispersed in suspension by sonicating in 10% serum in tissue culture medium. BEAS 2B cells were exposed to various particle concentrations (5 to 200 μg/ml) and cellular toxicity assessed by multiple colorimetric assays (WST-1, MTT, and LDH release) and direct counting methods (hemacytometer/trypan blue staining and flow cytometry/calcien AM and propidium iodine staining). ROFA exposure produced a dose dependent increase in BEAS 2B cellular cytotoxicity detected with comparable results by all colorimetric assays and direct cell counting methods. MTT and WST-1 colorimetric assays were found to produce significantly different results following exposure of BEAS 2B cells to all SWCNTs. Direct cell counting methods had to be employed to accurately assess the effect which various SWCNTs had on BEAS 2B cell growth\viability. Results from hemacytometer cell counting and LDH analyses showed a differential ability of SWCNTs to inhibit BEAS 2B cellular growth with no effect on viability or cytotoxicity. These results were confirmed by flow cytometry analyses. These studies show the ability of SWCNTs to inhibit BEAS 2B cellular growth was dependent on their production method. In addition, certain colorimetric assays cannot accurately assess the cellular effects of SWCNTs. Finally, SWCNTs may alter airway cell growth by mechanisms different from environmental combustion particles. (This abstract does not necessarily reflect EPA Policy)

1505 INHALATION EXPOSURE TO AEROSOLIZED CARBON NANOTUBES.

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Nanotechnology is a rapidly growing industry which is expected to provide great promise for advancement in fields of imaging, electronics, and therapeutics. Because of its unique electronic, optical, mechanical, chemical, or even biological

properties, carbon nanotubes (CNTs) are a nanomaterial that has gained notable attention for application in many of these industries. Due to the potential for human exposure in future occupational and consumer product use settings, toxicological studies are needed to understand the potential health hazards of these nanomaterials. Therefore, Sprague Dawley rats were exposed to aerosolized CNTs (200-300 μg/m3, purified or containing Fe catalyst) or fresh air via nose-only inhalation for 6 h for 1 d. To evaluate the inflammatory response in the lungs, cellular differentials were conducted on bronchoalveolar lavage (BAL) collected from rats following 0, 1, 3, and 7 d post-exposure (PE). Results showed that exposure to either purified or Fe-containing CNTs did not have a significant effect on the total number of lavaged cells or the number of macrophages, basophils, eosinophils and lymphocytes as compared to the controls. However, exposure to purified CNTs resulted in a significant increase in the number of monocytes following 7 d PE and exposure to Fe-containing CNTs caused a statistically significant increase in monocyte and neutrophil numbers immediately after 6 h exposure (0 d PE) and an increase in epithelial cell numbers following 7 d PE. Histological evaluations and mucin staining of lung tissue suggested changes in bronchiolar epithelium which were dependent on the type of nanoparticle, as well as the time period following exposure. While these data suggest that exposure to aerosolized CNTs may induce an inflammatory response in the lungs, further research is needed to evaluate whether these changes are transient or suggestive of precursor events to pathological changes that might develop under more severe exposure conditions or have implications for human health risk for persons potentially exposed to airborne CNTs.

1506 MUITIWALL CARBON NANOTUBE (MWCNT) EXPOSURE MONITORING IN A CNT RESEARCH LABORATORY BEFORE AND AFTER IMPROVEMENT OF WORKPLACE ENVIRONMENT.

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MWCNTs are expected to be applied in a wide range of industrial applications and consumer products. As production and wide use of MWCNT are growing, human and environmental exposure to MWCNT is likely to increase. We have monitored possible exposure of MWCNT-release in a MWCNT research laboratory. To estimate potential exposure to researchers, and to evaluate the improvement of workplace environment before and after installation of ventilation or other protective meaures, personal monitoring was conducted 4 sampling sites where workers handled unrefined materials. The number, composition and aspect ratio of MWCNT were measured by a scanning transmission electron microscopy with energy dispersive X-ray analyzer. The gravimetric concentrations of total dust before the improvement ranged from 0.03 to 0.43 mg/m3 and decreased to 0.031-0.039 mg/m3 after the installation of protective measures. The numbers of MWCNT from the sample obtained from the MWCNT mixer were ranged from 172.9-193.6 MWCNT/cc before the improvement and decreased to 0.008-2.0 MWCNT/cc after the improvement. Strikingly chrysotile asbestos fibers (0.01-1.3 fiber/cc) were detected in several work areas. The sources of asbestos were insulation materials used for MWCNT synthesis. The airborne asbestos concentration also decreased after the installation of protective measures. Our study suggested that ventilation or other protective measure can reduce exposure to airborne CNTs and other particulate materials in nano research facility.

1507 INHALED MULTIWALLED CARBON NANOTUBES POTENTIATE AIRWAY FIBROSIS IN A MURINE MODEL OF ALLERGIC ASTHMA.

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Asthma is exacerbated by inhalation of particulate matter in animal models and in humans, but the effects of inhaled, man-made nanomaterials on pre-existing lung disease are unknown. Multiwalled carbon nanotubes (MWCNT) are engineered nanomaterials with a high aspect ratio, a property shared with fibers known to cause fibrotic lung disease. This study tested the hypothesis that inhaled MWCNT would enhance airway remodeling and fibrosis in the ovalbumin-sensitized mouse model of allergic asthma. Control and ovalbumin-sensitized male C57/BL6 mice were exposed to 100 mg/m3 aerosolized multiwalled carbon nanotubes or vehicle for 6 h by nose-only inhalation. Animals were sacrificed 1 d and 14 d after exposure. Bronchoaveolar lavage fluid (BALF) and lungs were collected for histopathology and ELISA for cytokines. The majority of MWCNT delivered to the lungs was found in macrophages residing within the lung parenchyma and in the distal airways. Airway morphometric analysis revealed a significant ($p<0.01$) increase in airway fibrosis in sensitized mice exposed to MWCNT compared to sensitized mice treated with vehicle 14 d after exposure. However, inhalation of MWCNT had no effect on airway fibrosis in mice that were not sensitized. BALF levels of TGF-β1 and IL-13, cytokines associated with airway remodeling, were significantly elevated in ovalbumin-sensitized mice 24 h after inhalation of MWCNT or vehicle compared to similarly exposed mice that were not sensitized. These results are important for risk assessment, since the data indicate that individuals with pre-existing allergic airway disease may have increased sensitivity to airway fibrosis from inhaled MWCNT compared to normal populations. (Supported by The American Chemistry Council's Long Range Research Initiative provided to CIIT at the Hamner Institutes and by the College of Agricultural and Life Sciences at North Carolina State University).

1508 THE TOXICITY OF FRESH *VERSUS* AGED NANOPARTICLES.

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A number of factors can influence the pulmonary response to inhaled nanoparticles. We hypothesized that there will be a difference in the toxicity of fresh (predominantly singlet) vs. aged (predominantly agglomerated) metal nanoparticles and exposed inbred mice to filtered air or zinc and copper nanoparticles and examined lung injury and inflammation 24 hrs after exposure. We previously reported that freshly generated carbon nanoparticles (size: 10-50 nm) were more toxic than aged nanoparticles (size: 160-250 nm). In this study, fresh copper and zinc nanoparticles (size: 10-50 nm) were far more potent than similar size carbon in inducing lung injury and inflammation (e.g., significant inflammation and injury occurred at 5 mg/m3 carbon vs. 0.2 mg/m3 for copper and zinc nanoparticles). However, while there was a trend for greater toxicity in mice exposed to fresh copper and zinc nanoparticles versus aged nanoparticles, this difference was not as great as observed with carbon nanoparticles. These differences imply either differences in biochemical mechanisms between the carbon and metal nanoparticles or that physicochemical properties of fresh and aged nanoparticles are dependent on the manufactured nanomaterial type. We also examined whether host genetic factors play a role in the response to freshly generated zinc oxide nanoparticles. Significant inter-strain differences in response were observed amongst 20+ strains of mice exposed to 0.7 mg/m3 zinc oxide nanoparticles. A haplotype mapping computational algorithm was used to identify chromosomal regions that contained candidate genes responsible for the inter-strain differences in response. Thus, both physicochemical properties of nanoparticles as well as biological factors can be involved in the variability in response to inhaled nanoparticles.

1509 CARBON NANOTUBES AND BACTERIAL LIPOPOLYSACCHARIDE (LPS) ACT COORDINATELY TO STIMULATE PLATELET-DERIVED GROWTH FACTOR (PDGF) SIGNALING AND LUNG FIBROSIS IN RATS.

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Single- and multi-walled carbon nanotubes (SWCNT and MWCNT) have many technological applications, yet the health effects of nanotubes in humans remain to be elucidated. We previously reported that SWCNT cause lung fibrosis in rats and increase PDGF, a potent mitogen and chemoattractant for fibroblasts. We postulated that pre-exposure to LPS, a bacterial wall component that causes lung inflammation, would enhance PDGF signaling and lung fibrosis caused by SWCNT or MWCNT *in vitro* and *in vivo*. Sprague-Dawley rats pre-exposed to LPS (2.5 μg/ml) by nasal aspiration 24 hours prior and then treated with MWCNT (4 mg/kg) by intratracheal instillation had significantly higher lung fibrosis scores at 21 days post-exposure than rats receiving MWCNT alone. LPS alone caused no fibrosis. Immunostaining showed that macrophages located within fibrotic lesions caused by MWCNT contained PDGF. CB nanoparticles (8 nm) alone caused no lung fibrosis. LPS pre-exposure coupled with CB exposure caused fibrosis, albeit not to the same degree as LPS plus MWCNT. *In vitro*, q-PCR revealed that MWCNT or SWCNT (10 μg/cm^2) increased PDGF mRNA levels ~2-fold in primary rat lung fibroblasts after 24 hours, while LPS increased PDGF receptor (PDGFR) ~10-fold. SWCNT or CB nanoparticles did not increase PDGF mRNA levels in a rat alveolar macrophage cell line yet LPS priming (10 μg/ml) followed by SWCNT or CB increased PDGF mRNA levels ~80-fold and ~50-fold, respectively. LPS treatment alone increased PDGF mRNA levels ~8-fold. Therefore, LPS primes macrophages to produce PDGF in response to nanotubes and primes fibroblast responsiveness to PDGF by up-regulating PDGFR. These data support our hypothesis that LPS and carbon nanotubes coordinately stimulate PDGF signaling and lung fibrosis. (Supported by The American Chemistry Council, NCSU College of Agricultural and Life Sciences, and NIEHS)

1510 NANOPARTICLES SELECTIVELY STIMULATE NADPH OXIDASE IN RAT PULMONARY CELL LINES.

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Nanomaterials are being identified for a number of applications raging from cosmetics to circuitry. With the increase in production and use comes an increase in the potential for human and environmental exposures which when combined with an uncharacterized nanoparticle hazard presents a potential increase in risk. At the current time the mechanism of toxicity of engineered nanoparticles is not clearly understood. There has been some research suggesting that nanoparticles are capable of eliciting their damage via oxidative damage through the production of reactive oxygen species. This study addresses a possible mechanism of this toxicity; namely through induction of the NADPH oxidase complex. Rat derived pulmonary epithelial and endothelial cell lines were cultured and exposed to copper, titanium dioxide, silver and aluminum nanoparticles in the presence of a cell impermeant fluorescent dye(Amplex Red®) to assess the extracellular oxidant production following nanoparticle stimulation. Alveolar type I like and endothelial cell lines react to particle stimulation by increased production of oxidants while alveolar type II cells showed minimal response. To elucidate the role of NADPH oxidase in this system cells were incubated with pharmacologic inhibitors of NADPH oxidase or with agents known to disrupt the assembly of an active complex through disruption on membrane lipid rafts. We can conclude that nanoparticles possess an intrinsic radical producing activity which appears to be supplemented by cellular ROS emanating from the NADPH oxidase complex. These effects varied with both particle and cell type, underlining the importance of considering the system used to assess nanoparticle toxicity.
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1511 UPTAKE OF FINE AND ULTRA-FINE POLYSTYRENE LATEX BEADS IN A549 CELLS.

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Human exposure to nanomaterials is a potential public health concern due to their dramatic increased production and use in consumer products. Emerging research has demonstrated that nanoparticles are transported into cells; however, the consequences of this uptake as well as the mechanism of cellular uptake have not been fully elucidated. In this study, we used a type-II-like lung alveolar cell line (A549) to examine the mechanisms of uptake of ultrafine (26 nm) and fine size (250 nm) fluorescent polystyrene latex (PSL) beads. Following a 20 min in vitro exposure, flow cytometry and confocal microscopy reveal that uptake of the ultra-fine particles occurs via both temperature-dependent and temperature-independent pathways and that cellular uptake involves actin. Uptake of the fine particles is temperature-dependent, and involves actin and microtubule proteins. Clathrin and caveolae-dependent mechanisms do not appear to play a role in the internalization of either particle as pretreatment of the cells with chlorpromazine or low potassium and filipin, respectively, had no effect on uptake. Taken together, these data suggest that different endocytic processes mediated the uptake of the ultra-fine and fine PSL beads in A549 cells. These findings improve our understanding of the movement of nanomaterials across cell membranes and may provide insights into how these processes heighten their toxicity or improve their drug delivery capability.

1512 NANOPARTICLE DEPOSITION EFFICIENCY IN RAT AND HUMAN NASAL REPLICAS.

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Studies of particulate morbidity and mortality have suggested that airborne ultrafine particles (< 0.1 μm) may pose a greater hazard primarily due to their small size and potential to deposit in the deep lung. With increasing production of nanoparticles (materials deliberately produced in the size range of 1 to 100 nm), there is concern over the health effects of inhaled engineered nanoparticles. Measurements were made of the deposition efficiency of nanoparticles in replicas of one F344 rat and four healthy human nasal airways. Monodisperse nanoparticles of sucrose or diethylhexyl sebacate (DEHS) in the size range from 5 to 100 nm were produced using an electrospray aerosol generator (Model 3480, TSI, Inc., Shoreview, MN) or a nebulizer (one-jet Collison nebulizer CN241, BGI, Inc, Waltham, MA) with electrostatic classifier (Model 3080, TSI, Inc., Shoreview, MN). The aerosol was passed through a nasal replica in the inspiratory direction at a constant flow rate. The concentration of the nanoparticles was measured upstream and down stream of the nose model using a scanning mobility particle sizer (SMPS, Model 3934 or Model 3036, TSI, Inc., Shoreview, MN). The deposition fraction was determined from the difference in particle concentration between the upstream and downstream measurements. In general, for all of the replicas (1 F344 rat and 4 humans), the deposition efficiency increased as particle size decreased. For the 4 human nasal replicas, the deposition efficiency did not appear to depend on flow rate or nasal geometry. These results were consistent with diffusivity playing a major role in the deposition of particles in the nanometer size range. The results from this study have been used in computer models of particle deposition in the respiratory tract (See poster by B. Asgharian). This work was funded by US EPA STAR Grant #RD832531 and the Long Range Research Initiative of the American Chemistry Council.

1513 EFFECTS OF CERIUM OXIDE ON RAT PRIMARY ALVEOLAR MACROPHAGES.

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Diesel emissions pose health concerns in both environmental and occupational settings. The use of cerium as a fuel-borne catalyst lowers emission of carbonaceous diesel exhaust particles (DEP) and their hydrocarbon content, but results in the emission of ultrafine and fine cerium oxide particles. Due to its nanoparticle size, cerium oxide emission is a potential cause of health concern. Cerium oxide is a thermally stable strong oxidant and is capable of mediating cell-derived nitric oxide levels; thus, it may have a strong effect on alveolar macrophage (AM)-mediated immune/inflammatory responses. The objective of this study is to characterize the in vitro dose-dependent effects of cerium oxide (1.25 to 50 μg/ml) on rat AM functions in the absence or presence of DEP (50 or 100 μg/ml). AM were isolated from Sprague Dawley rats via bronchoalveolar lavage, cultured with cerium oxide, DEP or their combination for 24 h. Intracellular oxidants and mitochondrial damage of AM were determined using confocal microscopy. The particle-induced cytotoxicity and pro-inflammatory cytokine production by AM were monitored as lactase dehydrogenase (LDH) activity or the level of TNF-α and IL-12 in the culture media, respectively. The results show that cerium oxide induced LDH release, significantly blocked resting and LPS-induced nitric oxide and IL-12 production in a dose-dependent manner, but did not induce intracellular superoxide generation, mitochondria damage, or TNF-α secretion. Cerium oxide did not affect DEP-induced intracellular ROS or mitochondria damage, but significantly reduced DEP-induced IL-12, but not TNF-α, production at rest or in response to LPS. This study shows that direct interaction of cerium oxide with AM results in dose-dependent cellular damage and suppression of macrophage responses to LPS or DEP in the production of nitric oxide and IL-12, thus indicating a potential suppressive effect by cerium oxide on the innate and cell-mediated immunity against respiratory infection.

1514 METHODOLOGY FOR GENERATING NANOPARTICLE AEROSOLS- FROM BENCHTOP TO INHALATION LABORATORY.

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Issues surrounding occupational safety of workers exposed to nano-sized particles and materials are becoming increasingly pertinent in nanotechnology today. We have developed a novel approach to generating and characterizing a nanoparticle aerosol stream appropriate for testing, both in the workplace environment as well as for animal toxicity exposures. Although other methods are known to produce nano particulate matter, a limited number of techniques are available to synthesize fully characterized particle aerosols in the nano-size regime with precise control over size, size distribution, and concentration. The work described herein presents a proven method of producing amorphous nano-sized SiO2 particles via an aerosol nanoparticle reactor. Other particles consisting of different chemical composition can be synthesized as well (e.g., citric acid, TiO2, NaCl). The reactor is capable of synthesizing particles ranging d50 = 10 – 70 nm at concentrations ranging 10E4 – 10E7 particles/cm3. Characterization instrumentation in-line with the generation system allows for crystallinity (XRD), size and size distribution (DMA), and concentration (AE) measurements to be made in situ and upstream of the animal exposure chamber. This un-aggregated particle generation methodology enables subsequent testing with a continuous, stable supply of aerosol nanoparticles to facilitate inhalation toxicological profiles of animals exposed to aerosolized nanoparticle systems. This system was utilized in a short-term inhalation study with rats exposed to uncoated as-synthesized, amorphous SiO2 in two different nanoparticle size range populations (i.e. number distributions centered at d50 = 30 nm and d50 = 70 nm). The pulmonary toxicity results are described in a companion presentation.

1515 2, 3, 7, 8 TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) INHIBITS FIBROBLAST GROWTH FACTOR (FGF)10-INDUCIBLE PROSTATIC BUDDING IN THE MOUSE UROGENITAL SINUS (UGS).

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Numerous molecular signaling pathways collaborate to initiate prostate development by the UGS. FGF10, which is required for prostatic bud formation, is synthesized by UGS mesenchyme and elicits its signaling activity by binding to receptors in the UGS epithelium and activating ERK1/2-dependent mitogen-activated protein kinase signaling. TCDD inhibits ventral prostatic bud formation by the male fetal mouse UGS, culminating in ventral prostate agenesis. The purpose of this study was to test the hypothesis that TCDD impairs prostatic bud formation by inhibiting FGF10 signaling in the UGS. A UGS organ culture model was used to demonstrate that FGF10 recombinant protein increased prostatic bud number and that TCDD partially blocked this effect. *In utero* TCDD exposure (5 μg/kg, maternal dose) also delayed the normal activation of FGF10-dependent ERK1/2 phosphorylation in UGS epithelium by one day, from embryonic day (E)16-17. *In situ* hybridization was used to determine whether decreased expression of *Fgf10* or decreased expression of its receptor, *Fgfr2*, could account for the TCDD-dependent decrease in ERK activation. *In utero* TCDD exposure did not substantially alter the expression pattern or abundance of *Fgf10* or *Fgfr2*. Thus, the mechanism by which TCDD inhibits FGF10-dependent prostatic budding is dependent on interference with a molecular signal that lies downstream of FGFR2 activation, but upstream of ERK1/2 phosphorylation. (Supported by NIH grants F32 ES014284 and R37 ES01332).

1516 PROSTATIC BUDDING BY THE MOUSE UROGENITAL SINUS (UGS) IS INDUCED BY NOTCH SIGNALING; THIS EFFECT IS INSENSITIVE TO 2, 3, 7, 8 TETRACHLORODIBENZO-*p*-DIOXIN (TCDD).

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Mouse prostate development begins on embryonic day (E)16, when androgen-dependent prostatic buds emerge from the UGS in a pattern that establishes mature dorsolateral, anterior and ventral prostate lobe position. TCDD inhibits ventral prostatic budding and thus formation of ventral prostate. Retinoic acid (RA) and fibroblast growth factor 10 (FGF10) both induce prostatic budding in UGS organ culture and TCDD inhibits their bud inducing activity by a mechanism that cannot be explained by a direct action of TCDD on FGF10 or RA receptor abundance. The purpose of this study was to determine if NOTCH signaling is involved in prostatic budding and if it is sensitive to disruption by TCDD. Expression patterns of NOTCH signaling genes during the prostatic budding stage (E15-17) were determined by *in situ* hybridization. Expression of the NOTCH ligand jagged 1 (*Jag1*) was localized to a band of mesenchyme immediately adjacent to UGS epithelium and partially overlapped aryl hydrocarbon receptor expression. *Notch1* and its target gene, hairy and enhancer of split 1 (*Hes1*), were expressed predominantly by UGS epithelium. *In utero* exposure to TCDD (5 μg/kg maternal dose) on E15 did not greatly change *Jag1*, *Notch1*, or *Hes1* mRNA abundance on E16 or E17. UGS organ culture was used to determine whether activation of NOTCH signaling would support prostatic bud formation. Addition of a soluble recombinant form of JAG1 (JAG1-Fc) to UGS organ culture media nearly doubled prostatic bud number over the vehicle-exposed group, while both a NOTCH inhibitor (DAPT) and TCDD inhibited budding. Interestingly, however, induction of prostatic budding by JAG1-Fc was not sensitive to inhibition by TCDD. These results implicate NOTCH1 signaling in the mechanism of prostatic bud initiation and suggest that the molecular target for TCDD-mediated budding inhibition is downstream of RA and FGF10 but upstream of NOTCH1 signaling. (Supported by NIH grants F32 ES014284 and R37 ES01332).

1517 WNT5A REDUCES PROSTATIC BUD FORMATION IN THE MOUSE UROGENITAL SINUS (UGS) AND INHIBITION OF WNT5A RESCUES EFFECTS OF 2, 3, 7, 8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) ON BUD FORMATION IN VITRO.

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We have demonstrated a region-specific effect of TCDD on prostatic bud formation in the mouse whereby *in utero* exposure causes ventral prostatic bud agenesis while having lesser effects on budding in other regions of the UGS. As *Wnt5a* is expressed in the mouse UGS during budding, region-selective modulation of WNT5A signaling pathway activity was considered a potential mechanism by which TCDD inhibits ventral budding. Examination of *Wnt5a* knockout (KO) male mice revealed genital deformities of variable severity. 30% of animals exhibited agenesis of the bladder, UGS, and membranous urethra. Those that had formed a UGS exhibited hindgut-UGS fistulas and imperforate anus. When grafted to the renal capsules of male immunodeficient mice to assess prostate differentiation potential, *Wnt5a* KO male UGSs showed reduced ability to form mature ventral prostate compared to wild-type (WT) controls. *In vitro*, both TCDD and recombinant WNT5A protein (rmWNT5A) significantly reduced UGS prostatic bud formation, which is consistent with previous reports that WNT5A is an inhibitor of prostate morphogenesis in rodents. A WNT5A inhibitory antibody (aWNT5A-IgG) had no effect on vehicle-exposed UGSs but rescued inhibition of prostatic budding by TCDD. Further, when cultured UGSs were grafted under the kidney capsule of nude mice for 28 days, TCDD-exposed UGSs showed reduced ventral prostate-specific gene expression but aWNT5A-IgG + TCDD-exposed UGSs did not. While *in utero* TCDD exposure did not alter the abundance of *Wnt5a* mRNA or spatial *Wnt5a* expression in UGSs from WT male mice, these data support the hypothesis that TCDD inhibits ventral prostatic bud formation by increasing WNT5A activity in the ventral UGS. (NIH grants ES01332, HD049323, and ES014284)

1518 A TERATOLOGY STUDY OF ORAL GAVAGE OF LIU-WEI-TI-HUANG-WAN IN RABBITS.

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Liu-Wei-Ti-Huang-Wan is a traditional Chinese herbal medicine containing 6 herbs, Radix Rehmanniae preparata, Fructus Corni, Rhizoma Dioscoreae, Rhizoma Alismatis, Cortex Moutan, and Poria. The herbal extract has been widely used to improve the immune system function and female menopause syndromes. But the use of Liu-Wei-Ti-Huang-Wan during pregnancy has been questioned might cause adverse effect on the fetuses. The purpose of this study was to evaluate the effects of Si-Wu-Tang on maternal pregnancy, embryo-fetal developments and the fetuses in rabbits.

Two groups of mated New Zealand White rabbits were dosed daily during gestation days 6 to 18 via oral gavage with 300 mg/mL of Liu-Wei-Ti-Huang-Wan suspensions with the dosing volume of 10 mL/kg. Rabbits were observed daily throughout gestation days 6 to 29. The maternal body weights and food consumption were recorded at designated time intervals. At near term G29, the dams were euthanized and Cesarean sectioned. The number of viable or dead fetuses per litter, pre-implantation loss, and post-implantation death were recorded. External, visceral and skeletal abnormalities of fetuses were examined.

There were no maternal significant differences between control and dose groups in all parameters. Low incidences of spontaneous external, visceral and skeletal abnormalities of fetus were observed in both the treated and/or control groups.

The daily recommended human dose is 12-16 g for a 60-kg subject; therefore, the maximal daily recommended dose estimated is 267 mg/kg approximately. Based on the results, rabbits received dose up to 3000 mg/kg/day of Liu-Wei-Ti-Huang-Wan did not reveal any maternal toxicity and teratogenicity in the maternal, which provided a safety margin of 11.2 folds over the recommended human dose basing on a conversion of body weight. It is concluded that Liu-Wei-Ti-Huang-Wan is unlikely to induce detrimental embryo-fetal toxicity and teratogenicity in rabbits.

1519 EFFECTS ON THE CARDIOVASCULAR SYSTEM DURING A CONTINUOUS INTRAVENOUS INFUSION TERATOLOGY STUDY IN THE FEMALE RAT.

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The objective of this study was develop a model for continuous evaluation of blood pressure and heart rate during gestation, thus allowing improved assessment of drug-related effects on hemodynamic parameters in reproductive toxicology studies. Telemetry transmitters (DSI TA11PA-C40) were implanted subcutaneously into the flank and the femoral artery catheterized in female Sprague Dawley CD [Crl:CD(SD)] rats. Animals were left for one week to recover from surgery prior to mating. Mean arterial pressure, heart rate, systolic blood pressure, diastolic blood pressure and pulse pressure were measured using the Life Science Suite TM PO NE MAH Physiology Platform P3 software system (version 4.4) via Data Sciences International (DSI) Open A.R.T, continuously from Day 2 of gestation until Day 21 and animals were treated with continuous intravenous infusion of saline from Day 6 to 17.

Decreases in systolic, diastolic, mean and pulse pressures, of up to 20 mmHg relative to baseline, were noted for all animals from approximately Day 15 through Day 20. However no changes were noted for heart rate. Estrous cycles were unaffected relative to non-instrumented animals. Pregnancy rate was 100%, fetal weights were comparable with those from non-instrumented animals and there were no major malformations or minor external anomalies noted.

In conclusion, this model can be used for accurately assessing hemodynamic parameters in reproductive toxicology studies and there was no evidence of any adverse effects on the reproductive function secondary to the experimental procedures.

1520 VARIATION, LEVELS AND CONGENER PROFILES OF POLYBROMINATED DIPHENYL ETHERS (PBDES) IN HUMAN GESTATIONAL MEMBRANES.

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Polybrominated diphenyl ethers (PBDEs) have been incorporated into many consumer products as flame retardants. Due to their environmental stablity and ability to bioaccumulate, PBDEs have been quantified and reported in a wide array of human tissues from industrialized nations. However reports of PBDE levels in human reproductive tissues have remained limited and incomplete, focusing only on fetal blood and tissues. Preliminary data from our laboratory suggests PBDEs act as inflammatory agents in extraplacental gestational membranes (amnion, chorion, deciduas), as measured by PBDE-stimulated cytokine release in human explants and cell cultures. The aim of the current study is to evaluate the congener specific profiles of PBDEs from human gestational membranes. Gestational membranes from five term pregnancies were obtained from non-laboring caesarian deliveries at the University of Michigan Women's Hospital Birth Center. Replicate samples were extracted and analyzed by GC-MS for 22 congeners and composites. The majority of the total PBDE loading was due to BDEs 47, 49, 99, 100, 153. No octa- or deca- BDEs were identified in this analysis. BDE 49 comprised nearly 20% of the total PBDE level but has not previously been reported in reproductive tissue assessments. This work provides the necessary baseline information needed for in vitro evaluation of PBDEs impact on inflammatory processes in gestational tissues.

1521 MONO-2-ETHYLHEXYL PHTHALATE STIMULATES RELEASE OF THE PROINFLAMMATORY CYTOKINE INTERLEUKIN-6 IN HUMAN GESTATIONAL MEMBRANE CULTURES.

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Mono-2-ethylhexyl phthalate (MEHP) is an active metabolite of di-2-ethylhexyl phthalate (DEHP), a ubiquitous chemical pollutant primarily used as a plasticizer in the manufacturing of polyvinyl chloride (PVC) products and as a fragrance stabilizer in personal care products. Because elevated levels of the proinflammatory cytokine interleukin-6 (IL-6) are found in the amniotic fluid of women who deliver preterm and MEHP stimulates release of the proinflammatory cytokine IL-6 in human lung epithelial cells, we evaluated if MEHP stimulates release of IL-6 in human gestational membranes in vitro. With IRB approval, extra-placental membranes were obtained from healthy non-laboring term pregnancies delivered by cesarean section. Gestational membrane punches consisting of amnion, chorion and decidua were cultured in media containing dimethyl sulfoxide (DMSO; solvent control), or 25, 50, 100 and 200 μM MEHP for 24 h. Lipopolysaccharide (LPS; 100 ng/mL) was used as a positive proinflammatory control. Secretion of IL-6 into membrane media was measured by enzyme immunoassays. To confirm ELISA results, immunohistochemical analysis of IL-6 in gestational membrane sections was performed. IL-6 release in membrane media was significantly increased by LPS, 25, 100 and 200 μM MEHP treatments. This was verified by immunohistochemical analysis of IL-6 in membrane sections following stimulation with 200 μM MEHP or 100 ng/mL LPS. Previous studies have linked infection-stimulated inflammation with preterm birth. The current study shows that MEHP can act as a non-infectious proinflammatory stimulus to release IL-6 in human gestational membranes in vitro. These results suggest that MEHP may warrant further investigation in epidemiologic studies of preterm birth.

1522 *IN UTERO* EXPOSURE TO BENZENE CAUSES STRAIN AND GENDER SPECIFIC CHANGES IN HEMATOPOIETIC PROGENITOR CELLS.

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Chronic exposure to benzene is associated with an increased risk of developing leukemia. Furthermore, epidemiological studies show that in utero exposure to benzene is correlated with an increased incidence of childhood leukemia. In utero exposure to inhaled benzene has been shown to alter erythroid hematopoietic progenitor cell numbers in fetal and neonatal Swiss Webster mice with differences between males and females. Our objective was to determine if in utero exposure to benzene i.p. altered erythroid as well as myeloid progenitor cell numbers and determine if these changes were strain and/or gender specific. Pregnant C57Bl/6N and CD-1 dams were injected i.p. on gestational days (GD) 8,10,12, and 14 with either corn oil (vehicle) or benzene (200 mg/kg, or 400 mg/kg). GD16 fetal and 2-day-old neonatal liver tissue was collected and plated in Methocult media containing appropriate cytokines. Erythroid CFU-E and BFU-E colonies, and myeloid CFU-G, CFU-M, and CFU-GM were incubated for 3-12 days, and then counted. Our results show a strain and gender difference in progenitor cell sensitivity to in utero benzene exposure. In C57 GD16 fetuses, in utero exposure to 200 mg/kg benzene significantly increased male and female BFU-E and CFU-G colonies, male CFU-E, and female CFU-GM colonies. The 400 mg/kg treatment group showed a significant increase in male CFU-E colonies only. Conversely, female GD16 CD-1 fetuses showed a significant increase in CFU-E colonies after in utero exposure to 200mg/kg benzene. C57 2-day-old neonates did not show a significant change in colony number in any of the progenitor cell types tested. However, CD-1 2-day-old neonates showed a significant decrease in female CFU-E and male CFU-M colonies within the 200 and 400 mg/kg treatment groups, respectively. Together these results support the hypothesis that in utero benzene exposure significantly alters numbers of hematopoietic progenitor cells in fetal and neonatal tissue and these alterations are strain and gender specific. [Support: CIHR]

1523 EFFECT OF MATERNAL FOLIC ACID SUPPLEMENTATION ON VALPROIC ACID INDUCED TERATOGENESIS IN CULTURED MOUSE EMBRYOS.

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Exposure to the anticonvulsant drug valproic acid (VPA) in utero is associated with a 1-2% increase in neural tube defects (NTDs), however the molecular mechanisms by which VPA induces NTDs is unknown. Although perinatal folic acid supplementation reduces the risk of NTDs by 60-70% in the general population, it is unclear whether it protects against the teratogenic effects induced by VPA. Several studies have shown prevention of VPA-induced NTDs by folic acid in mouse models in vivo. The objective of this study is to evaluate the maternal and embryonic components of this embryoprotection in CD-1 mice. Pregnant dams were injected with folinic acid (FA) (4 mg/kg) or vehicle (PBS) three times daily starting on gestational day (GD) 5.0 through to GD 8.0. On GD 9.0, embryos were extracted and those with 4 to 6 somites were exposed to VPA (0 or 0.60 mM) and FA (0, 1, or 2.5 mM) in a whole embryo culture system. Protection against exencephaly was observed in embryos extracted from dams pretreated with FA and cocultured with VPA and FA at 1 and 2.5 mM. Co-culture of a high concentration of FA (2.5 mM) and VPA with embryos extracted from PBS treated mice also provides embryoprotective effects against VPA-induced exencephaly. Preliminary results indicate that FA protection against VPA-induced NTDs is embryo specific since maternal pretreatment alone was not embryoprotective, and high concentrations of FA can negate the teratogenic effects induced by VPA. With the establishment of an embryo culture model to examine these protective effects, the mechanism by which FA protects against VPA-induced teratogenesis will be investigated. Future directions will involve measuring global methylation changes in VPA and FA exposed embryos using the cytosine extension assay, as well as monitoring the methylation status of specific genes using bisulfite DNA sequencing. (Support: CIHR).

1524 EXPLORING THE DEVELOPMENTAL EFFECT OF THALIDOMIDE AND IRRADIATION ON LIMB PATTERNING.

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Thalidomide is a mild-sedative initially produced from 1957 until 1961. By the early 60's it had become increasingly clear that administration of thalidomide in women was responsible for the dramatic increase in specific birth defects such as absence of ears, heart defects and various limb deformities, the most prevalent and striking of which was phocomelia (lack of proximal limb structures). While the tragedy of thalidomide led to the re-evaluation of drug safety standards, it also became a riddle for developmental biologists who aimed at understanding how thalidomide could alter patterning, and more specifically limb patterning, so dramatically.
In the present study, we propose to investigate the effect of thalidomide as well as irradiation, which also leads to phocomelia, from a developmental stand-point. Specifically, we will examine limb morphology and vascularization as well as cell death and proliferation in exposed limbs. We will also look at the expression of different markers of proximo-distal identities as well as broad gene expression. We first

examined cell death and expression of markers of specific identities along the proximo-distal axis in irradiated chick limbs. We found that 24 hours post-irradiation, exposed chick limbs showed an increase in apoptosis as well as a decrease of expression of Meis-1, a marker of proximal fate. By contrast, the distal markers Hoxa11 and Fgf8 were not affected. These results suggest a sensitivity of proximal determining pathways to irradiation. We are also examining cell proliferation, markers of intermediate fate as well as the integrity of the vasculature following irradiation. We will conduct similar experiments in rabbits following thalidomide exposure. By comparing the effect of both irradiation and thalidomide, we hope to deepen our understanding of how proximo-distal patterning is established during normal development and how it is affected by teratogens.

1525 DEVELOPMENTAL TOXICITY OF DIMYRCETOL IN RATS.

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Dimyrcetol (CAS 18479-58-8, 25279-09-8), a widely used fragrance ingredient, was evaluated for developmental toxicity in Crl:CD(SD) rats. Groups of 25 presumed-pregnant rats were intubated (gavage) with 0, 250, 500, or 1000 mg/kg/day dimyrcetol in corn oil on gestation days (GDs) 7-17. Dams were observed for viability, clinical signs, body weights, and feed consumption. On GD 21, dams were necropsied and Caesarean-sectioned. Corpora lutea were counted and uteri were examined for implantations, fetuses, and resorptions. Live fetuses were weighed and examined for gender and gross external changes. One-half of the fetuses per litter were examined for soft tissue or skeletal alterations. No maternal deaths or treatment-related clinical signs were observed. The 1000 mg/kg/day dosage produced maternal body weight losses in the first two days of treatment which resulted in reduced maternal body weights for the entire dosage period and were associated with significant reductions in feed consumption. Associated threshold developmental toxicity observed at 1000 mg/kg/day consisted of reversible delays in development including a 3% reduction in fetal body weights, reversible retardation in ossification of the metatarsal bones, occurring at values that were within the historical ranges of the testing facility and not considered toxicologically important, and an increase in supernumerary thoracic ribs, with associated increases and decreased in thoracic and lumbar vertebrae, respectively, a minor common variation that also is reversible and frequently observed at maternally toxic dosages. Based on the maternal body weight losses and reduced maternal feed consumption and reduced fetal body weights and increases in reversible variations in skeletal ossification, maternal and developmental NOELs of 500 mg/kg/day and NOAELs of 1000 mg/kg/day were established for dimyrcetol. Dimyrcetol was not selectively toxic to development and did not cause fetal malformations, even at a maternally toxic dosage.

1526 REPRODUCTIVE AND DEVELOPMENTAL RISK ASSESSMENT DATA IN CYNOMOLGUS MONKEYS OF CAMBODIAN, CHINESE, AND INDONESIAN ORIGIN.

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The cynomolgus monkey (*Macaca fascicularis*) is a useful animal model for preclinical reproductive and developmental toxicity studies of biotechnology-derived therapeutics. Even though cynomolgus monkeys of Chinese origin have been commonly used for these types of studies, it is essential to establish alternative sources of animals. This will ensure adequate availability to meet the ever increasing demand for this species.

The goal of this paper is to categorize similarities and differences in embryo-fetal development parameters between cynomolgus monkeys of different origin. Pregnancy ratios in Chinese (CH) and Cambodian (CA) animals were 40.9 and 49.2%, respectively [no data available for Indonesian (IN) animals]. Incidences of abortion and embryo-fetal death (7.5 - 9.8%), fetal external abnormalities (0.0-1.6%), placental changes (11.2-23.1%), visceral abnormalities (0.0-1.6%), skeletal abnormalities (0.0-3.2%), and skeletal variations (13.2-23.1%) were comparable in animals of the three origins. Fetal weight, placental weight, and fetal external and skeletal measurement parameters in CA and IN fetuses were within ±10% of the fetuses obtained from CH fetuses, except for female anogenital distance in CA (89%) and tail length in IN (113%). Fetal organ weights were varied between these three origins, and many organs in Indonesian fetuses were heavier than CH fetuses. Only thymus, spleen, and mesenteric lymph node weights were obtained in CA fetuses. Both thymus and spleen weights in CA fetuses were comparable with those of the CH fetuses while the mesenteric lymph node was heavier.

In conclusion, control background data including pregnancy rates (except for Indonesian animals), spontaneous abortion ratio, fetal abnormalities/variations, and fetal external and skeletal measurements data were comparable between cynomolgus monkeys of different origin. There were some variations in fetal organ weights, especially between CA/CH and IN animals.

1527 DEVELOPMENTAL TOXICITY OF METHYL DIHYDROJASMONATE (MDJ) IN RATS.

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Methyl dihydrojasmonate (MDJ, CAS # 24851-98-7) is a widely used fragrance ingredient. It has a warm, sweet-floral, jasmine-like and fruity odor. It was evaluated for developmental toxicity in Crl:CD(SD) presumed-pregnant rats (25/group) at oral dosages of 0, 40, 80 or 120 mg/kg/day in corn oil administered on gestational days (GDs) 7-20. Dams were observed for clinical signs, body weights, viability and feed consumption. Dams were necropsied and Caesarean-sectioned on GD 21, corpora lutea were counted, and the uteri were examined for number and distribution of implantations, live and dead fetuses and resorptions. All live fetuses were weighed and examined for gender, gross external changes, and one-half of the fetuses per litter were evaluated for soft tissue or skeletal alterations. No maternal deaths occurred. MDJ-related observations at 120 mg/kg/day included adverse clinical signs (an increased incidence of sparse hair coat and ungroomed appearance), gross necropsy observations (two dams tan areas in the liver and a pale spleen), reduced mean maternal body weight gains and body weights during the dosage period and reduced absolute and relative maternal feed consumption for the entire dosage period. No Caesarean-sectioning or litter parameters were significantly affected by dosages of MDJ as high as 120 mg/kg/day, although there was a tendency toward slightly reduced mean fetal body weight at the 120 mg/kg/day dosage. No fetal gross external, soft tissue or skeletal changes were attributable to dosages of MDJ as high as 120 mg/kg/day. Based on these data, a maternal No-Observable-Adverse-Effect-Level (NOAEL) of 80 mg/kg/day and a developmental NOAEL equal to or greater than 120 mg/kg/day were established for MDJ. Hence, under the conditions of this study, MDJ was not a selective developmental toxicant in rats and did not cause fetal malformations at a maternally toxic level.

1528 EARLY RESPONSE IN GENE EXPRESSION PROFILES IN MONKEY EMBRYOS FOLLOWING MATERNAL EXPOSURE TO THALIDOMIDE DURING THE SUSCEPTIBLE PERIOD FOR MALFORMATIONS.

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The congenital limb defects that characterized the human abnormality have been observed and thereafter replicated in monkeys. In order to understand molecular mechanisms underlying the detrimental effects associated with thalidomide teratogenicity, altered gene expression profiles were determined in the embryos of pregnant cynomolgus monkeys given thalidomide during the susceptible period for thalidomide-induced malformations. The cynomolgus monkeys were given with (±)-thalidomide at 15 or 20 mg/kg/day by oral administration on days 26-28 of pregnancy, and fetuses were examined on day 80 of pregnancy. Limb defects, such as micromelia/amelia, paw/foot hyperflexion, polydactyly, syndactyly, and brachydactyly, were observed in seven of the eight fetuses. To determine early response in gene expression in whole embryos maternally exposed to thalidomide, pregnant cynomolgus monkeys were orally given thalidomide at 20 mg/kg on day 26 of pregnancy, and whole embryos were removed from the dams six hours after administration. Three embryos each in the thalidomide-treated and control groups were obtained. Total RNA were isolated from individual embryo and amplified. cRNA was hybridized to the cynomolgus monkeys gene chip (Affimetrix) and, normalization was performed. A total of three embryos were individually analyzed to detect the thalidomide responsive genes. A total of 50 up-regulated genes and 242 down-regulated genes were 1.5 folds changes for all of thalidomide-treated embryos compared with those of controls. The selected genes including focal adhesion, regulation of actin cytoskeleton, aminoacyl tRNA biosynthesis, cell cycle and signal transduction related genes. These genes were uniquely and early responding to thalidomide in embryos of cynomolgus monkeys.

1529 DEVELOPMENTAL TOXICITY OF N, N-DIMETHYLPROPANAMIDE IN RATS.

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N,N-Dimethylpropanamide (DMPA) and N,N-dimethylacetamide (DMAA) are industrial solvents. DMAA has been shown to cause fetal malformations in rats and is classified as an developmental toxin (Repr. Cat. 2; R61 – may cause harm to the

unborn child) in the EU. As both solvents are structurally closely related, a developmental toxicity screening study was performed with DMPA to evaluate whether DMPA might induce comparable effects.
Mated female rats (8/group) were given 0, 225, 450 and 700 mg/kg bw/day DMPA on gestation days 6-20 by oral gavage. Clinical signs, food consumption and body weight development were recorded. All rats were C-sectioned on gestation day 21 and macroscopically examined. The number of corpora lutea was determined and the uteri weighted. The fetuses were sexed, weighted, examined for gross external abnormalities and killed. At least one half of the fetuses of each litter was processes for evaluation of visceral malformations, the remaining fetuses were processed for evaluations of skeletal malformations.
All dams survived until scheduled necropsy. Body weight gain and food consumption were dose dependently and statistically significantly decreased at all dose levels. All dams at 450 and 700 mg/kg had total embryonic resorptions. At 225 mg/kg mean fetal weights were significantly decreased (-23%). This was accompanied by a statistically significantly higher incidence of non-ossified bones suggesting a delay of development. Visceral examination of the fetuses revealed an increased incidence of heart interventricular septal defects, dilated arch and narrowed ductus arteriousus. These findings are comparable with those reported for DMAA.
Even though the fetal effects were observed at a maternally toxic dose, it is concluded that the incidence and type of malformations found in the present study indicate a teratogenic potential of DMPA. Based on the presented data, the teratogenic potency of DMPA seems to be comparable to DMAA. A NOEL could not be established in the present study.

1530 ANALYSIS OF ETHANOL-INDUCED ABNORMALITIES IN THE MEDIAL FOREBRAIN OF FETAL MICE.

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Although prenatal ethanol exposure is the leading known cause of congenital central nervous system (CNS) dysfunction, much remains unknown regarding the full spectrum of ethanol-induced defects or their developmental basis. The focus of the current study was to examine the effect of early prenatal ethanol exposure on the developing forebrain in a mouse model for fetal alcohol syndrome (FAS). For this work, gestational day (GD) 7 C57Bl/6J mouse embryos were acutely exposed to ethanol via 2 maternal 2.9 g/kg intraperitoneal injections. Control and treated specimens were either collected on GD 12.5 for histological analysis and *in situ* hybridization or on GD 17 for high resolution magnetic resonance imaging studies. For the latter, the fetuses were immersion fixed in a Bouins/Prohance solution. 512X512X1024 image arrays were acquired using a 7-Tesla magnet and employed for regional CNS segmentation, 3-D reconstruction and volume assessments. Illustrated was a spectrum of CNS abnormalities, with major sites of deficiency being the rostral and ventro-medial forebrain; i.e. the septal region, olfactory bulbs and striata of GD17 fetuses. On GD12.5, the ventro-medial loss was evidenced histologically by either abnormally close proximity of the ganglionic eminences (GE) or loss of tissue in this structure. *In situ* hybridization utilizing Nkx2.1 to selectively identify the medial GE is being employed to determine the specificity of effects on this tissue. Additionally, immunohistochemical analyses directed toward identifying effects on specific GE-derived populations, esp. oligodendrocytes and inhibitory interneurons, are in progress. Ethanol-induced reductions of the GE-derived cell populations could explain, in part, the behavioral problems in children with FAS.
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1531 ORAL (GAVAGE) DEVELOPMENTAL TOXICITY STUDY OF 4-TERTBUTYLCYCLOHEXYL ACETATE (4-TBCHA) IN RATS.

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A developmental toxicity study was conducted to detect any potentially adverse effects of 4-tBCHA, a widely used fragrance ingredient. One hundred Crl:CD(SD) pregnant rats (25/group) were administered 4-tBCHA or corn oil (vehicle) by gavage at doses of 0, 40, 160, or 640 mg/kg/day once daily on gestation days (GDs) 7 through 20. All rats were observed for clinical effects, abortions, premature deliveries, and deaths. Body weights and feed consumption values were recorded. The rats were sacrificed on GD 21, necropsied, and Caesarean-sectioned. All live fetuses were weighed and examined for sex and gross external alterations. Approximately one-half of the fetuses in each litter were examined for soft tissue or skeletal alterations. Adverse effects observed at the 640 mg/kg/day dosage level included sacrifice of one rat with severe adverse clinical effects (all others survived to GD 21), statistically significant increases in moderate excess salivation, red perioral substance, sparse hair coat on the limbs, and increased incidences of extreme excess salivation, ungroomed coat, and sparse hair coat and localized alopecia of the limbs and/or neck. Also, the 640 mg/kg/day dose significantly reduced maternal body weight gains, body weights, and absolute and relative feed consumption values. Developmental observations at 640 mg/kg/day consisted of transient retardations in fetal development (significant reductions in fetal body weight and associated significant increases in moderate dilation of the renal pelvis, delayed ossification of the caudal vertebrae, fore- and hind-limb phalanges and hind-limb metatarsals). No other Caesarean-sectioning or litter parameters were affected by 4-tBCHA as high as 640 mg/kg/day. On the basis of this data, both the maternal and developmental no-observable-adverse-effect-levels (NOAELs) for 4-tBCHA are 160 mg/kg. 4-tBCHA is not a selective developmental toxicant in rats and did not produce fetal malformations, even at a maternally toxic dosage.

1532 LOW-DOSE TRICHLOROETHYLENE EXPOSURE DURING VALVULOSEPTAL MORPHOGENESIS CAUSES VENTRICULAR SEPTAL DEFECTS IN HATCHED CHICKS.

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Trichloroethylene (TCE) is a common groundwater contaminant in the U.S. Several studies associate gestational TCE exposure with congenital heart defects; however, this conclusion is controversial and the basis for TCE's cardiac teratogenicity is not well understood. We previously showed that chick embryos exposed to an in ovo concentration of ~8 ppb TCE during valvuloseptal morphogenesis have reduced cardiac output, hyperplastic cushions and increased mortality; exposure during the earlier cardiac specification period had little adverse effect. To further narrow this critical window, we exposed in ovo embryos during valvuloseptal morphogenesis to a single TCE level (mean egg concentration 0.4 – 4000 ppb) applied at a single developmental time point (stages 13, 15, 17, 20 or 24; 2-4.25 days' incubation). Stages 15 and 17 were the most sensitive to TCE and those embryos had <57% survival to stage 30 (6.5 days' incubation), suggesting that the early cushion period represents the critical window of TCE sensitivity. These embryos had a biphasic dose response to TCE with the greatest mortality at ~8 ppb, a level just above the maximum contaminant level for TCE in human drinking water. Exposure at the other stages had lesser effects on mortality. Hatched chicks exposed to ~8 ppb TCE at stage 17 had a significant incidence (38%; $p<0.01$; n=16) of high ventricular septal defects (VSDs) as detected by transthoracic echocardiography and confirmed by dissection; control injected chicks had no VSDs (n=14). These data independently confirm that TCE is a cardiac teratogen for chick and identify early valvuloseptal morphogenesis as a critical window for this common groundwater contaminant. (Supported by NIH award ES11738 and T32 ES07015)

1533 A STUDY OF THE EFFECTS OF GREEN TEA CATECHINS (GTC) ON EMBRYO/FETAL DEVELOPMENT IN RATS.

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The beneficial health effects associated with the consumption of green tea are widely recognized and claimed to be due to tea catechins. Heat treatment of marketed green tea beverages for sterilization causes epimerization and/or polymerization of tea catechins. Although several studies have been conducted on the physiological functions of tea catechins, studies on the safety of heat-treated tea catechins are limited. The objective of the present study was to evaluate potential adverse effects of green tea catechin preparations for use in beverages that have undergone heat sterilization (GTC-H). GTC-H was administered to rats orally at levels of 200, 600 and 2000 mg/kg/day during the critical period of organogenesis. GTC-H, in the vehicle, deionized water, was administered by gavage to three groups of 25 bred female Crl:CD®(SD) rats once daily from gestation days 6 through 17. Dosage volumes for all groups were 10 mL/kg. The only test article-related clinical finding was hair loss on various body surfaces in the 2000 mg/kg/day group. Lower mean maternal body weight gains, net body weight gains, and food consumption were observed at dose levels of 600 and 2000 mg/kg/day. Mean gravid uterine weights and intrauterine growth and survival were not affected by GTC-H at any dose level. No GTC-H related fetal malformations or developmental variations were noted in this study. The lower mean body weight gains and food consumption in the 600 and 2000 mg/kg/day groups were likely related to the caffeine content in GTC-H, as previous studies have demonstrated maternal toxicity following oral administration of caffeine to rats. Based on the results of this study, a dose level of 200

mg/kg/day was considered to be the no-observed-adverse-effect level (NOAEL) for maternal toxicity and a dosage level of 2000 mg/kg/day was considered to be the NOAEL for embryo/fetal development.

1534 PRENATAL AND POSTNATAL EFFECTS IN RATS OF PERFLUORONONANOIC ACID EXPOSURE *IN UTERO*.

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In recent years, our understanding of the developmental toxicity of perfluoroalkyl acids (PFAAs) has focused on the C8 compounds. Ubiquitous environmental contaminants, PFAA's are utilized as surfactants in a variety of industrial applications. Although several PFAAs have been extensively studied, the paucity of data for perfluorononanoic acid (PFNA) led us to conduct the current study. An initial range-finding study was conducted with timed pregnant Sprague-Dawley (SD) rats dosed by oral gavage with 0, 1, 10, or 30 mg/kg PFNA from gestation day (GD) 2-21. On GD 8, rats dosed with 30 mg/kg PFNA were euthanized due to overt maternal toxicity. Three of seven rats in the 10 mg/kg PFNA dose group were also euthanized on GD 11 due to excessive toxicity. Remaining dams in the 10 mg/kg group had reduced maternal body weight gain throughout gestation and reduced pup weights, compared to controls. Based on these initial findings, a larger study was conducted with timed pregnant SD rats dosed with 0, 1, 3, or 5 mg/kg PFNA from GD 2-21 (n=12 per group). Maternal body weight gain and food intake were monitored throughout gestation. On GD 21, Caesarean sections were performed on half of the dams. Maternal body weight, percent live fetuses per litter, and litter size and weight were similar between the groups. However, dams exposed to 5 mg/kg PFNA had significantly higher liver weights compared to controls (11%). The fetuses were examined for external malformations but none were observed. Remaining dams were allowed to deliver and were monitored for pup survival, weight gain, and postnatal development. Postnatally, pups exposed to 5 mg/kg PFNA had significantly lower birth weight than controls (16%), which remained lower than controls through early postnatal development. Postnatal survival through day 7 was not affected by PFNA exposure. These data indicate that exposure of pregnant rats to PFNA may elicit developmental toxicity at doses of 5 mg/kg or above. This abstract does not necessarily reflect EPA policy.

1535 EMBRYOTOXICITY OF RETINOIC ACID IN POSTIMPLANTATION RAT WHOLE EMBRYO CULTURE MONITORED WITH GENE EXPRESSION ANALYSIS.

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Reproductive toxicology is the most animal-consuming area in toxicology. This relates to the necessity to perform prenatal and multigeneration studies for human risk assessment. In order to minimize animal use and also to determine mechanisms of embryotoxicity, toxicogenomics approaches could be used in postimplantation whole embryo culture (WEC) as a screening system for hazard identification. WEC is a validated alternative developmental toxicity test which allows the in vitro assessment of effects of chemicals on organogenesis. In this study, all-trans-retinoic acid (RA), a model embryotoxic compound, was used to assess the potential of toxicogenomics to define biomarkers of developmental toxicity in WEC. RA-mediated effects include malformation of limbs, loss of anterior structures including the forebrain, most of the midbrain and eyes, whereas the hindbrain and spinal cord appear to expand in compensation. Using WEC, gestation day 10 WU rat embryos (2-5 somites) were exposed for 4 hours to 0.5 µg/ml retinoic acid. Total embryonic RNA was harvested and used in a microarray analysis. In addition, embryos were exposed for 48h to determine morphological effects. We observed significant changes in the expression of several RA-responsive genes. These include genes associated with brain development, and patterning of rhombomeres and limb buds. We conclude that the toxicogenomics approach used delivered several genes that could be used as biomarkers for embryotoxicity. This study demonstrates the feasibility of gene expression analysis in WEC as a tool for embryotoxicity assessment.

1536 AN EVALUTION OF DEVELOPMENTAL LANDMARK, SENSORY AND REFLEX TOXICITY TESTING AND CORRELATION TO PUP BODY WEIGHT IN CRL:CD(SD) RATS.

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Currently, the extent and method of data collection for developmental, reflex and behavioral testing in pre- and postnatal developmental studies (Segment III) varies in the industry based on the interpretation of the ICH Harmonized Tripartite Guidelines. The ICH Harmonized Tripartite Guidelines states that the best indicator of physical development is body weight and the achievement of preweaning landmarks of development, sensory functions, reflexes and other behavioral parameters are highly correlated with neonate/pup body weight. At Covance Laboratories Inc., eleven different maturation parameters are measured over a range of days in which developmental changes are taking place, and include pinna unfolding, hair growth, incisor eruption, eye opening, surface righting reflex, auditory startle, pupil reflex, learning and memory, locomotor activity, vaginal opening and preputial separation. Here, we determined whether delays in maturation parameter achievement correlate with body weight. Data cumulated from Segment III studies conducted from 1996 and 2007 were assessed for delayed maturation parameters and compared to neonate/pup bodyweight at the time of 100% litter achievement. The results indicated that delays in physical development, as well as sensory and reflex parameters, are highly correlated with significantly decreased neonate/pup bodyweight. These data suggest that body weights proved to be the most sensitive indicator of delayed development. In some cases, significant decreases in body weights were noted without a delay in the maturation landmarks. No change in any single development landmark parameter impacted the NOEL or NOAEL. In conclusion, these data can be used to define the current industry standard for maturation parameter testing.

1537 TWO-GENERATION ASSESSMENT OF REPRODUCTIVE TOXICITY AND TERATOGENICITY OF A NOVEL CALCIUM/POTASSIUM SALT OF (-)-HYDROXYCITRIC ACID.

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(-)-Hydroxycitric acid (HCA), a natural extract from the dried fruit rind of Garcinia cambogia, has been demonstrated to serve as a novel weight-loss supplement. This two-generation study was conducted to evaluate the effects of a hydroxycitric acid extract (HCA-SX, commercially known as Super CitriMax) on the reproductive systems of male and female rats, the postnatal maturation and reproductive capacity of their offspring. Across two generations, Sprague Dawley rats (30/sex/group) were maintained on feed containing HCA-SX at dose levels of 0, 1000, 3000, or 10,000 ppm for 10 weeks prior to mating, during mating, gestation and lactation. Clinical toxicity signs, body weight and feed consumption were recorded. For the parents (F0 and F1) and the offspring (F1 and F2a), reproductive parameters such as fertility, mating, gestation, parturition, litters, lactation, sexual maturity and development of offspring were assessed. At termination of the study, necropsy and histopathological examinations were performed. HCA-SX supplementation to parental male and female rats of both (F0 and F1) generations during the premating and mating periods, for both sexes, and during gestation and lactation in case of female rats, did not reveal any remarkable frequency of mortality or abnormal clinical signs. HCA-SX exposure did not affect feed consumption, body weight, or reproductive performance as evaluated by sexual maturity, fertility and mating, gestation, parturition, litter properties, lactation and development of the offspring. The parental as well as the offspring no-observed-adverse-effect level (NOAEL) for HCA-SX was thus determined to be greater than 10,000 ppm in diet or equivalent to 1018 and 1524 mg/kg body weight/day in male and female rats, respectively. This study in conjunction with earlier studies reconfirmed the safety of HCA-SX for human consumption.

1538 DEVELOPMENTAL TOXICITY ASSESSMENT OF A NOVEL CALCIUM/POTASSIUM SALT OF (-)-HYDROXYCITRIC ACID.

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(-)-Hydroxycitric acid (HCA) is obtained from the rind of Garcinia cambogia fruit. HCA is known to affect fat synthesis, reduce food intake, and has been used as a novel therapeutic tool in weight management. The teratogenic potential of a HCA extract (HCA-SX, commercially known as Super CitriMax) was investigated in a multi-generation reproduction toxicity model. Sprague Dawley rat pups (~30/sex/group) were selected randomly from the F2b generation and were grown to 10-12 weeks before mating to start the teratology study. HCA-SX exposure was conducted through feed, while the weaning rats had exposure through lactation. The dietary exposure levels of HCA-SX were 0 (control), 1000, 3000, or 10,000 ppm. Following mating at maturity, the pregnant rats were observed daily for clinical signs, body weight and feed consumption. On day 20th of the gestation, animals were subjected to necropsy and caesarean section to examine the uterus,

ovaries and fetuses for assessment of different parameters of pregnancy and embryo-fetal defects which included death, structural anomalies and altered or retarded growth. A slight (13%) lowering of maternal body weight gain during gestation period in the group receiving 10,000 ppm HCA-SX was observed. However, there was no evidence of any adverse effects on the parameters evaluated for the maternal toxicity, gravid uteri, external abnormalities, soft tissue abnormalities, or skeletal abnormalities in the fetuses. Overall, HCA-SX was found to be non-teratogenic in Sprague Dawley rats, at the dietary exposure levels up to 10,000 ppm, equivalent to 1240 mg/kg body weight/day, respectively. The present study demonstrates the broad spectrum safety of HCA-SX.

1539 POST-TRANSLATIONAL MODIFICATION OF P53 DURING STRESS-INDUCED RELEASE FROM THE MITOCHONDRION.

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Induction of p53 tumor suppressor protein accumulation is an early response to low-level stressors and can be suppressed in the early mouse embryo with PK11195, an embryo-protective ligand of the mitochondrial peripheral-type benzodiazepine receptor (Bzrp/TspO). When recombinant p53-EGFP fusion protein is targeted to the mitochondria in p53-null MEF cells via the COVIIIL signal peptide, mitochondria react to low-level chemical stressors by releasing p53 to the cytosol through a PK11195-sensitive process. Recombinant p53 then translocates to the nucleus. To test the hypothesis that mitochondrion-derived p53 has unique biochemical properties, the present study has examined post-translational modifications of p53 in p53-null MEF cell cultures. The cells were transfected with expression vectors for recombinant p53-EGFP fusion protein led by a sense (competent) or antisense (incompetent) mitochondrial import signal. The products of these vectors are designated m53-EGFP and c53-EGFP, respectively. Chemical hypoxia induced with rotenone (100nM,1h) invoked the translocation of m53-EGFP from mitochondrion to nucleus, thus shifting MEF cells from Class-0 (mitochondrial) to Class-2 (nuclear) p53-EGFP states. Preliminary findings utilizing immunofluorescence and western blotting techniques with antibodies directed against specific sites for p53 serine phosphorylation and lysine acetylation suggest changes in post-translational status may accompany the redistribution of p53. For example, p53 phosphorylation at serine 15 occurred only after release from the mitochondrion. We are currently looking for mitochondrion-specific changes; however, the preliminary data suggest that release of p53 from mitochondria is part of a signaling cascade that is initiated by mitochondrial dysfunction and resembles the DNA damage response. The extent to which this mechanism might account for embryo-protective effects of PK11195 is under investigation. (Supported by NIH grant AA13205).

1540 THE RABBIT IS NOT AN APPROPRIATE DEVELOPMENTAL TOXICITY TEST SYSTEM FOR BACTERIOSTATIC IRRITATIVE COMPOUNDS SUCH AS THE FUNGICIDE FOLPET.

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Folpet is a fungicide that acts to inhibit germination of spores by chemically reacting with thiol (-SH) groups. The degradation process generates two main entities: thiophosgene and phthalimide (PI). Thiophosgene is transient; PI, by contrast, is stable. In an effort to elucidate the mode of action for maternal toxicity that is seen with folpet, Minimum Inhibitory Concentration (MIC) assays were conducted with folpet and PI. The basis for this work is the importance of cecotropes for proper nutrition of does. Bacteria, resident in the cecum, are responsible for fermenting otherwise non-digestible material and, in the process, generate fatty acids, amino acids, and vitamins. Folpet, in MIC assays, showed marked antimicrobial activity towards all three species with MIC values in the range of 20-50 μg/ml for Bacteroides sp., 50 - 500 μg/ml for Enterococcus faecalis, and ≤ 2-5 μg/ml for Candida albicans. In contrast, PI showed MIC values in excess of 1000 μg/ml. These data suggest that folpet can disrupt the intestinal flora of does and, in doing so, may contribute to maternal toxicity. Direct irritation of the gastrointestinal tract, evident in dietary studies with mice, may also be a component of maternal toxicity. The presence of maternal toxicity, unrelated to the direct developmental toxicity of test materials, confounds the interpretation of study data from materials that are bacteriostatic and intestinal irritants. These studies provide a cautionary note when planning developmental toxicity studies in rabbits with test materials that are irritative, bacteriostatic, or both.

1541 EFFECTS OF SUNITINIB, AN ANGIOGENESIS INHIBITOR, ON EMBRYO-FETAL DEVELOPMENT IN THE RAT AND RABBIT.

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Introduction: Angiogenesis plays a critical role in physiological processes, including embryo-fetal development (EFD) and maturation. We report the results of three studies on the effects of orally administered sunitinib, a multitargeted RTK inhibitor of VEGF and PDGF receptors with antitumor and antiangiogenic activity, on EFD in female rats and rabbits.
Methods: Sunitinib was administered po qd to pregnant Sprague-Dawley rats and New Zealand White rabbits for 12 and 14 days, respectively, during organogenesis, at doses of 0 (control) to 0.3–30 mg/kg/day, and to 0.5–20 mg/kg/day, respectively. Clinical observations were recorded. Animals were humanely euthanized and cesarean section was performed on gestation days 20/21 (rats) and 29 (rabbits). Each uterus was weighed and a gross necropsy was performed. The numbers of live/dead fetuses, early/late resorptions, corpora lutea and total implantations were recorded. Fetuses were examined, weighed, and euthanized.
Results: In rats, mortality occurred at the highest dose, 30 mg/kg/day, and ≥5 mg/kg/day resulted in decreased gravid uterine weights, number of viable fetuses, and fetal body weight, plus an increased number of resorptions, and complete litter loss. Fetal malformations, primarily skeletal, occurred at >3 mg/kg/day; acephalostomia also occurred in 2 fetuses at 5 mg/kg/day. In rabbits, mortality occurred at the highest dose, 20 mg/kg/day, which was stopped early due to overt toxicity. At 5 mg/kg/day, reductions in gravid uterine weights and number of live fetuses were due to increased number of resorptions and complete litter loss. Cleft lip and/or cleft palate were observed at ≥1 mg/kg/day.
Conclusions: As expected for an angiogenesis inhibitor, sunitinib was associated with EFD toxicity in female rats and rabbits at or below the systemic exposure observed in clinical trials of sunitinib at 50 mg/day.

1542 MICROCYSTIN-LR CROSSES THE PLACENTA IN CD-1 MICE.

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The cyanobacterium *Microcystis aeruginosa* produces hepatotoxic microcystin-LR (MCYS). MCYS is a tumor promoter. The long term effects of prenatal MCYS exposure may include increased susceptibility to liver disease or cancer in postnatal life. To assess potential fetal microcystin uptake after long-term maternal exposure, pregnant CD-1 mice were implanted with subcutaneous pumps containing tritiated dihydromicrocystin-LR (release rate, 10μg/kg/day) on gestational day 15. Four days later, mice were euthanized and tissues isolated. Tissues were digested, ^{3}H dpm was measured, and equivalents of MCYS/g tissue were calculated. Also, to assess fetal MCYS content after acute maternal exposure, pregnant CD-1 mice were gavaged with tritiated dihydromicrocystin-LR (200 μg/kg) on gestational day 18. From 2-48 hr later, mice were euthanized and tissues analyzed as above. Following maternal exposure by pump (N=4), tritium was detected in maternal blood (5.8±3.0 ng MCYS equivalents/g tissue (MEQs) (±SD)) and liver (11.3±2.5 MEQs). MCYS was present in fetuses (9.8±1.4 MEQs) and placentas (12.1±1.3 MEQs). Following maternal exposure by gavage (N=2-4 each timepoint), tritium was detected in maternal blood (3.9±4.0 MEQs at 2 hr; 17.0±17.5 MEQs at 48 hr) and liver (4.7±2.6 MEQs at 2 hr; 13.9±10.0 MEQs at 48 hr). Again, MCYS was present in placentas (9.1±0.2 MEQs at 2 hr; 15.6±10.3 MEQs at 48 hr) and fetuses (5.6±3.2 MEQs at 2 hr; 8.9±3.4 MEQs at 48 hr). Unexpectedly, spleen tissue contained high MEQs at the earliest timepoint and remained elevated at later times (35.5±37.5 MEQs at 2 hr; 12.4±1.3 MEQs hr at 48 hr). When pups were born before sacrifice, maternal milk contained 0.7, 5.6, 6.4, and 17.9 MEQs. Fetal uptake of MCYS occurs following both long-term and acute exposure of pregnant female mice. Continuing studies will relate maternal dose to fetal dose. (Research supported by Florida Dept of Health and NIH PO1-ES51094-07.)

1543 ROLE OF FERROUS SULFATE IN HEXAVALENT CHROMIUM-INDUCED CYTOTOXICITY AND APOPTOSIS IN HUMAN DERMAL FIBROBLASTS.

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In the US, approximately 1.5 million construction workers are occupationally exposed to cement each year. Allergic contact dermatitis (ACD) is a major skin problem reported among cement workers. Hexavalent chromium [Cr(VI)], present as a contaminant in the cement, has been attributed to be responsible for cement ACD.

A significant reduction in the incidence of cement ACD has been reported in certain European countries where the addition of ferrous sulfate (FeSO4) to cement has been mandated. The role of FeSO4 to counteract cement ACD has been questioned and in the US the practice of addition of FeSO4 to cement to prevent ACD is not followed. Presently, we have conducted in vitro cell culture experiments to investigate whether FeSO4 is capable to protect cells of human dermal origin against Cr(VI)-induced toxicity. Human dermal fibroblasts were treated with potassium dichromate (0-50 μM final concentrations) alone or with potassium dichromate and FeSO4 (0, 10, 20 and 40 μM final concentrations) for 24-hours. At the end of the exposure period, cytotoxicity and apoptosis were determined in the cells by MTT assay and TUNNEL assay, respectively. Exposure of the fibroblasts to chromium alone resulted in a concentration-dependent cytotoxicity and apoptosis. The chromium-induced cytotoxicity and apoptosis in the fibroblasts were significantly blocked by the addition of FeSO4 to the culture medium. Similar protective effect was noticed when the experiments were repeated with human keratinocytes. In summary, our results demonstrated a protective role for FeSO4 in Cr(VI)-induced cytotoxicity and apoptosis in human dermal fibroblasts and keratinocytes. The findings may thus indicate that the incidence of cement ACD among construction workers in the US may be prevented by the addition of FeSO4 to the cement.

1544 REPEATED OPEN APPLICATION TESTS FOR ALLERGIC CONTACT DERMATITIS USING TWO CHEMICALS CONTAINING HEXAVALENT CHROMIUM.

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Hexavalent chromium [Cr(VI)] causes allergic contact dermatitis (ACD). While some studies have investigated Cr(VI)-induced ACD under patch-test conditions, occlusive conditions are not representative of environmental or occupational exposures. We conducted repeated open application tests (ROATs) in Cr(VI)-allergic individuals using two chemicals containing Cr(VI): potassium dichromate (PD) and acid copper chromate (ACC). All participants were patch-tested to confirm their Cr(VI) allergy and graded as 1+ (n=33), 2+ (n=11), or 3+ (n=16). ROAT challenges were conducted in 60 allergic and 10 non-allergic participants using five doses—0, 90, 250, 750, and 2,500 ng Cr(VI)/cm^2—and 10 applications on the forearms over two weeks. Twenty and 17 Cr(VI)-sensitized participants responded to PD and ACC, respectively. Dose-response data showed dose-related trends for allergic responses to PD and ACC. Of the participants who had allergic responses during ROAT, 59-65% had a patch-test grade of 3+, and 35% were 2+. The majority of the participants who had grade 2+ (55-64%) and grade 3+ (63-81%) patch test responses reacted during ROAT, whereas only one participant with 1+ patch-test grade responded. A few participants also had irritant responses to PD and ACC.
In summary, allergic responses occurred to both forms of Cr(VI), PD and ACC, and showed clear dose-related trends. Most of the ROAT responses occurred in participants who had a patch-test grade of 3+. These participants were the only ones who responded to the lowest dose, were the earliest to respond to all doses, and had the strongest ROAT responses. The dose-response data from these studies can be used to set protective standards, such as skin reference dose, for dermal exposures to Cr(VI) in treated wood or contaminated soil. The United States Environmental Protection Agency's Human Studies Review Board (HSRB) approved these studies for use in the dermal risk assessment of Cr(VI).

1545 VESICANT INJURY INCREASES EXPRESSION OF MATRIX DEGRADING ENZYMES AND INFLAMMATORY MEDIATORS IN A MOUSE EAR MODEL.

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Cutaneous exposure to sulfur mustards induces inflammation followed by the emergence of fluid-filled blisters and tissue damage. We used a mouse ear model to assess mechanisms of vesicant-induced injury. Treatment of mouse ears with the half mustard 2-chloroethyl ethyl sulfide (CEES, 65 μmol) was found to cause a time-dependent increase in skin inflammation and edema which was evident within 6 h. This was associated with marked increases in expression of MMP-9 and TIMP-1, two important mediators of extracellular matrix remodeling. Expression of the pro-inflammatory cytokine IL-1β, and vascular cell adhesion molecule-1 (VCAM-1), which mediates leukocyte extravasion to sites of tissue inflammation also increased. CEES treatment was also associated with increased mRNA expression for the antioxidant heme oxygenase-1 and enzymes important in the production of inflammatory mediators including inducible nitric oxide synthase, cyclooxygenase-2, and 5-lipoxygenase. These responses were correlated with rapid (within 6 h) increases in epidermal prostaglandin E2 and leukotriene B4, two eicosanoids that mediate the inflammatory response. These data indicate that matrix degrading enzymes and inflammatory mediators are altered following CEES exposure, a response that may contribute to the toxicity of a similar vesicant, sulfur mustard. Support: NIH CounterACT Program NIAMS U54AR055073. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the federal government.

1546 DEVELOPMENT OF TOXIC INDUSTRIAL CHEMICAL-INDUCED CUTANEOUS LESIONS IN SWINE.

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Due to continued public concern for safety from chemical attacks, the Battelle Biomedical Research Center (BBRC) and the U.S. Army Medical Research Institute of Chemical Defense (USAMRICD) have aligned research efforts to establish a cutaneous exposure time for a toxic industrial chemical (TIC) that produces both superficial dermal and deep dermal cutaneous injuries in an anesthetized weanling pig model. Liquid bromine was evaluated in female Yorkshire-cross pigs with four lesion sites (each 3 cm in diameter) between the axillary and inguinal areas of the ventral abdomen. Injury was produced by modifying exposure time to undiluted liquid bromine and evaluating the sites after 24 and 48 h. Lesion assessment included clinical observations, digital photographs, size measurements, modified Draize scoring, reflectance colorimetery, evaporimetry, and infrared thermography. Histopathologic evaluation of skin collected at 48 h post-exposure included wound severity, wound depth, and percent of the total area involved . A 45 sec exposure to 600 μL of bromine produced a superficial dermal lesion and an 8 min exposure produced a deep dermal lesion. The development of exposure techniques and selection of exposure times, as well as histopathologic characterization of injuries created by a TIC in a weanling pig model, consistently resulted in uniform lesions. Follow-on efforts will address efficacy testing of therapeutic compounds to improve wound healing by preventing/reducing the erythema, edema and deep tissue damage to the skin caused by exposure to a TIC like liquid bromine.
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1547 SKIN IMMUNOSENESCENCE: DECREASED RACK-1 EXPRESSION IS ASSOCIATED WITH DEFECTIVE TNF-ALPHA PRODUCTION IN RESPONSE TO DIFFERENT STIMULI.

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Skin immunosenescence may account for the increased susceptibility in the elderly to cutaneous infections and malignancies, decreased or variable contact hypersensitivity reactions and decreased response to vaccination.
We have recently shown that a decreased expression in the receptor for activated C kinase (RACK-1) underlies defective protein kinase C (PKC) activation and functional immune impairment with aging. Although an age-associated decrease in cutaneous immune cell number and function has been reported, relatively little is know about the molecular mechanism(s) underlying such defects. The present study was designed to determine if an age related decline in RACK-1 expression was present and whether it would be correlated with defective TNF-α production. Skin obtained from old rats (> 18 m) showed decreased RACK-1 immunoreactivity in both epidermis and dermis if compared to young rats (< 3m), as assessed by Western blot analysis and immunohistochemistry. RACK-1 preferentially interacts with PKC β. Despite a similar total skin content of this isoform, the reduced expression of RACK-1 was associated with a decreased translocation of PKC β in the membrane compartment.

The defective PKC β translocation correlated with decreased TNF-α release from epidermal cells following treatment with different inflammatory stimuli.
The defective TNF-α production is likely to significantly contribute to skin immunosenescence, as decreased production of this promigratory signal affects the vigour of Langerhans cells mobilization from the epidermis and compromises the initiation of cutaneous immune response.

1548 MEASUREMENT OF SKIN THICKNESS IN CONTROL SD RATS AND BEAGLE DOGS FROM TWO SUBCUTANEOUS TOXICITY STUDIES.

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The thickness of epidermis, dermis and hypodermis of control SD rats from a 26-week subcutaneous toxicity study was measured in an attempt to understand the implications of variable skin thickness on the incidence and distribution of vascular injection site lesions observed during this study. Similar measurements were recorded in control beagle dogs in a 26-week toxicity study. Skin sections from 20 rats/sex and 3 dogs/sex were evaluated morphometrically at all injection sites used in the studies. Each site was injected once in dogs and up to 2 times in rats with sterile saline. For each site (rat) or ipsilateral pair of sites (dog), the average thickness was calculated for each animal and the average variable was submitted to 2-sided statistical analysis. Data from each sex were evaluated separately. Differences in thickness of epidermis, dermis and hypodermis (the skin layer in which the compound was deposited) were observed craniocaudally in rats of both sexes; differences in dogs were limited to the hypodermis in both sexes. In rats, the epidermis and dermis of males were thicker and the hypodermis was thinner at all injection sites compared to females. In rats, all skin compartments were thickest at the thoracic region. In males, the epidermis and dermis were thinnest at the flank region, while the hypodermis was thinnest at the scapular region. In females, the epidermis was thinnest at the flank region and the dermis and hypodermis were thinnest at the scapular region. In dogs, no statistical differences were observed in epidermal and dermal thickness, but the hypodermis was thickest at the two most anterior and posterior injection sites. In conclusion, injection sites used for dorsal subcutaneous injection of xenobiotics in rodents and dogs are not equivalent. This, along with the volume and formulation of the compound injected, may influence the occurrence of injection site lesions and make their interpretation difficult regarding relevance for human safety.

1549 LIGAND ACTIVATION OF PPARβ/δ INHIBITS PROLIFERATION OF HUMAN HACAT KERATINOCYTES.

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The role of peroxisome proliferator-activated receptor-β (PPARβ) in cell proliferation is unclear. While there is strong evidence that ligand activation of PPARβ induces terminal differentiation and attenuates growth, some studies suggest that PPARβ potentiates cell proliferation via protein dependent protein kinase 1 (PDPK1) and protein kinase B (Akt). The present study examined the effect of ligand activation of PPARβ in HaCaT cells, a human keratinocyte cell line, using two highly specific PPARβ ligands and the putative PPARβ ligand retinoic acid (RA). Treatment with GW0742 and GW501516 led to inhibition of cell proliferation in the presence or absence of serum. Both highly specific PPARβ ligands led to a dose-dependent increase in mRNA for PPARβ target genes adipocyte differentiation related protein (ADRP) and angiopoietin-like 4 (Angptl4), but no change in PDPK1 expression was observed. Consistent with the lack of change in PDPK1 expression, ligand activation of PPARβ did not alter expression or phosphorylation of Akt. While serum withdrawal increased poly ADP-ribose polymerase (PARP) cleavage, ligand activation of PPARβ did not alter PARP cleavage. Since recent evidence suggested that RA is a PPARβ ligand, the effect of RA was also examined in primary mouse keratinocytes and HaCaT cells. RA altered mRNA expression of cytochrome P450 26A1 (CYP26A1) and transglutaminase 1 (Tg1), but did not effect Angptl4 expression in HaCaT cells. RA increased mRNA expression of CYP26A1 in mouse keratinocytes, and this effect was not mediated by PPARβ. Consistent with the lack of a PPARβ-dependent effect by RA, no change in mRNA expression of Angptl4 was found in mouse keratinocytes. Results from these studies demonstrate that ligand activation of PPARβ inhibits cell proliferation, consistent with a large body of evidence from independent laboratories. Further, results from these studies suggest that retinoic acid does not activate PPARβ in mouse or human keratinocytes.

1550 PERTURBATION OF GLUTATHIONE DETOXIFICATION PATHWAYS MAY BE INVOLVED IN BUTYLATED HYDROXYTOLUENE (BHT) MEDIATED POTENTIATION OF 4-TERTIARY BUTYLPHENOL (4TBP) TOXICITY IN MELANOCYTIC CELLS: IMPLICATIONS FOR OCCUPATIONAL VITILIGO.

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The exact mechanism of 4TBP mediated melanocyte destruction in the cause of occupational vitiligo is unclear. BHT, a commonly used antioxidant potentiates the cytotoxic potential of 4TBP in melanocytic cells. In this study the effect of (a) pretreatment with 100μM BHT for 10 days (b) depletion of glutathione (GSH) by 1mM buthionine sulfoximine (BSO) (c) inhibition of γ-glutamyltranspeptidase (GGT) by 1mM acivicin (ACV) and (d) BHT and 4TBP on GST activities on 4TBP toxicity in SK-MEL-23 (M23) and SK-MEL-19 (M19) melanoma cells lines was investigated. Cells maintained in DMEM (10% bovine serum, 1% antibiotic/antimycotic) were seeded in 96 well plates (2 X 105 cells/well) and allowed to attach overnight. After 24h, cells were exposed to various treatments, at the end of which toxicity was assessed by CellTiter 96 AQueousOne Solution Cell Proliferation Assay according to manufacturer's instruction. For GSH depletion and GGT inhibition studies, cells were treated with BSO or ACV for 6 hours prior to treatment with BHT & 4TBP. GST activity was determined using 1-chloro-2,4-dinitrobenze as substrate in lysates obtained from untreated cells or after 24h exposure to BHT, 4TBP, BSO and ACV respectively. BHT pretreatment for 10days did not potentiate 4TBP toxicity. In contrast to BSO, ACV treatment increased sensitivity of cells to 4TBP toxicity (M23>M19). Although pretreatment with BHT, 4TBP and BSO had no effect, ACV decreased GST activities in both cell lines. In in-vitro experiments, BHT (not 4TBP) significantly ($p<0.05$) inhibited GST activity in lysates of untreated cells. The principal conclusions are (a) both BHT & 4TBP must coexist for potentiation of toxicity (b) potentiation is most likely due to inhibition of GST activity and (c) inhibition of GGT but not depletion of GSH influences 4TBP toxicity in melanocytic cells.

1551 DIFFERENTIAL RESPONSES OF HEMIZYGOUS *VERSUS* HOMOZYGOUS TG.AC MICE TO THE PHOTOCARCINOGENIC EFFECT OF ULTRA-VIOLET RADIATION.

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The Tg.AC transgenic mouse model was developed by introducing into FVB mice a fused transgene consisting of the mouse embryonic zeta (ζ)-globin gene, activated *v*-Ha-*ras* and an SV40 polyadenylation splice sequence. It has been demonstrated that female homozygous Tg.AC mice develop papillomas in a dose-dependent manner as early as 4 weeks following exposure to ultraviolet radiation (UVR); however, the phototumorigenic response of hemizygous Tg.AC mice has not been demonstrated and was investigated in this study. Hair was clipped from the dorsal skin surface of 20 week old female homozygous and hemizygous Tg.AC mice and later exposed to a cumulative UVR dose (51% UVB; 49% UVA) of 22 kJ/m2 on three alternate days. Papillomas were evident as early as 4 weeks post UVR exposure in homozygous Tg.AC mice, in agreement with earlier published studies. Papilloma multiplicity increased from an average of 0.6 papillomas per mouse on week 3 to 1.3 papillomas by week 10, whereas a previous study demonstrated an average of 6 papillomas per homozygous Tg.AC mouse by week 10. In contrast, hemizygous Tg.AC mice exposed to UVR did not show a significant tumor response with the average papilloma per hemizygous Tg.AC mouse exposed to UVR being equivalent to that of non-exposed hemizygous mice with an average of 0.04 papillomas on week 4 post UVR exposure and 0.07 by week 10 post UVR. This study demonstrates that the hemizygous Tg.AC mice may be less sensitive the photocarcinogenic effects of UVR when compared to homozygous Tg.AC mice.

1552 DIFFERENTIATION OF DERMAL IRRITATION POTENTIAL OF ESTERS FOR SKIN CARE PRODUCTS.

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Skin care products and cosmetics are often intended for repeated, chronic dermal application. Therefore, they need to be formulated to minimize dermal irritation potential. Dermal irritation is objectively demonstrated by erythema and/or edema. However, even the slightest, subclinical irritation may prove unacceptable to the consumer. Prior to formulation, data are necessary to characterize and differentiate

the raw ingredient irritation profile to identify potential issues. In this study, comparative irritation data were generated for a set of esters to aid in the development of new personal care products. Esters are employed as skin conditioning agents. Although typically low in irritancy, they were selected because they are prevalent in a wide variety of skin care products. To detect slight differences in irritation potential, exaggerated exposure conditions were utilized. A 14-day cumulative irritation occlusive patch study was conducted with the undiluted esters. No significant objective dermal irritation was found with C12-C15 alcohols benzoate, stearyl heptanoate and polyglyceryl-4-isostearate. Octyldodecyl neopentanoate, propylene glycol dioctanoate and caprylic/capric triglycerides produced slight, delayed erythema in a small fraction of the subjects tested. Cetearyl octanoate and C12-15 alcohols octanoate elicited the most significant irritation responses of those tested. Interestingly, the log Kp, permeability coefficient, is the highest for these two esters suggesting that dermal penetration is a factor in their irritation potential. In conclusion, we were able to differentiate the dermal irritation potential of this set of esters. Although other factors and ingredients affect the overall irritation potential for the complete skin care product, this information can help select esters for the development of new skin care formulations or provide insights into improving an existing formulation.

1553 METABOLIC ACTIVITY OF SKIN *IN VITRO* SYSTEMS.

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The skin (integumentum commune) is a major barrier for many external substances. The species dependent differences in skin morphology are well studied, whereas less is known about the metabolic capacity of the skin. Albeit cutaneous first pass metabolism plays a role in barrier function of the skin by metabolic detoxification as well as metabolic toxification to cause skin toxicity and allergies.
The aim of this study was to estimate the metabolic activity of skin in vitro systems from different species. Human, pig and rat skin sub-cellular fractions of the epidermis were analyzed for their CYP- and GST-activity. Additionally, the metabolism of diagnostic substrates was analyzed. Similarities and differences of species will be presented and discussed in the context of available literature (Ösch F. et al., Drug Metabolism Reviews, accepted for publication).
Ethoxyresorufin-O-deethylase activities in skin microsomes were approximately 0.4 pmol resorufin /min/mg protein. Whereas Pentoxyresorufin-O-depentylase activity was approximately 0.4 pmol resorufin /min/mg protein in human skin microsomes, but were near the detection limit for rats and mini pigs. GST activities were comparable for rat and pigs (approximately 25 nmol 1-chloro-2,4-dinitrobenzene /min/mg S9-fraction) and 5-fold less as compared to human activities (approximately 130 CDNB /min/mg S9-fraction). The metabolism of the diagnostic substrates correlated with the characterized in vitro system properties.
In summary, human exhibit the highest metabolic capacity for xenobiotic metabolizing enzymes as compared to rat and mini pig. In contrast to their similar morphology the metabolic activities differed between human and pig skin.

1554 *IN VIVO* AND *IN VITRO* DERMAL ABSORPTION OF CREOSOTE.

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Coal tar creosote, a complex mixture of more than 200 compounds, is a wood preservative pesticide used to pressure treat railroad crossties, marine and foundation piling, and utility poles. The dermal penetration and absorption of creosote in vivo in the rat and in vitro in rat and human skin has been evaluated. Creosote was spiked with eight 14C-radiolabeled PAH's, which represented about 43% of the composition of creosote. In vivo, the spiked sample was applied to a 10.5 cm2 area, demarcated by glass O-ring glued to the shaved back, at a rate of 10 µL/cm2 to two groups of 4 rats. At the end of an 8-hour exposure, excess creosote was removed by washing and one group of rats was sacrificed and a second group was held for 21 days post-exposure. At sacrifice, the application site was tape-stripped to remove the stratum corneum and the total distribution of applied radioactivity determined for each animal. Following the 21-day period, 92.7% of the applied dose was recovered. Total absorption was 8.86%, which included a negligible portion of the applied dose that remained in the tape-stripped skin (0.005%). The unabsorbed portion of the applied dose, removed at 8 hours, was contained in skin wash (22.2%), body wrap (34.2%) and O-ring (27.5%). In vitro, with the same regimen of dose and exposure time, creosote penetrated through rat skin faster (85.3 µg equiv/cm2/h) than through human skin (19.7 µg equiv/cm2/h); total penetration (receptor fluid only) was greater for rat skin (665.8 µg equiv/cm2) than for human skin (149.7 µg equiv/cm2). Washing skin at the end of the exposure removed 12.8% and 70.3% of the applied creosote test substance from rat and human skin, respectively. The amount of dose associated with the stratum corneum was greater for rat skin (23.6%) than for human skin (5.33%). The total absorbable dose, receptor fluid plus any dose remaining in tape-stripped skin, was 34.3% and 4.24% for rat and human skin, respectively. Based on the prescribed application rate and exposure time, human in vivo dermal absorption to creosote was calculated to be 1.1%.

1555 *IN VITRO* HUMAN SKIN PENETRATION OF THE FRAGRANCE MATERIAL LINALOOL.

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The in vitro human skin penetration of linalool [CAS# 78-70-6] was assessed in three vehicles relevant to fragrance exposure [1:3 ethanol:diethyl phthalate (1:3 EtOH:DEP), 1:3 ethanol:dipropylene glycol (1:3 EtOH:DPG) and petrolatum]. The assays were conducted under both occlusive and non-occlusive conditions. Diffusion cells were prepared with epidermal membranes obtained from surgical donors. Permeation of linalool, from a 5 µl/cm^2 target dose of a 4% (w/v) solution, was measured at twelve time-points over 24 hours, using a phosphate buffered saline receptor phase. At 24 hours, the epidermal membranes were wiped, tape stripped 10 times and the linalool content of the wipes, strips and remaining epidermis determined. The filter paper skin supports were extracted and the diffusion cell donor chambers washed and wiped. Potential evaporative loss of linalool was estimated by measuring the loss from PTFE sheets under the same experimental conditions. Levels of linalool in the epidermis (plus any remaining stratum corneum after tape stripping), filter paper membrane support and receptor fluid were combined to produce a total absorbed dose value. Under non-occlusive conditions 2.71 ± 0.2% of the applied dose was absorbed at 24 hours in 1:3 EtOH:DEP, 2.01 ± 0.2% in 1:3 EtOH:DPG and 3.5 ± 0.4% in petrolatum. Under occlusive conditions 7.3 ± 0.58% of the applied dose was absorbed at 24 hours in 1:3 EtOH:DEP, 12.1 ± 1.5% in 1:3 EtOH:DPG and 10.5 ± 1.1% in petrolatum. Evaporative loss was significant and accounted for the differences in absorption between occluded and non-occluded conditions. From these results, systemic exposure to linalool from use in fragrances would be expected to be low based on currently reported use levels. In addition to providing information to risk assessments, the variety of vehicles will provide comparative data to assist in the development of dermal absorption models for this material.

1556 USE OF A MULTI-FIBER APPROACH TO QUANTIFY CHEMICAL MIXTURE INTERACTIONS MODULATING DERMAL ABSORPTION.

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Chemical mixtures can potentially increase or decrease systemic bioavailability of toxicants in a mixture. Dermal permeability can be modulated by changes in physicochemical interactions between mixture, skin and the solute of interest. These physicochemical interactions can be described as changes in system coefficients associated with molecular descriptors described by Abraham's linear solvation energy relationship (LSER). This study evaluated the effects of chemical mixtures containing either a solvent (ethanol) or a surfactant (sodium lauryl sulfate, SLS) on solute permeability and partitioning by quantifying changes in system coefficients in skin and a three membrane coated fiber (MCF) system, respectively. Regression analysis demonstrated that changes in system coefficients in skin were strongly correlated (R^2 = 0.89 – 0.98) to changes in system coefficients in the 3-membrane MCF array with mixtures containing either 1% SLS or 50% ethanol. The PDMS fiber appeared to play a significant role (R^2 = 0.84 – 0.85) in the MCF array in predicting changes in solute permeability, while the WAX fiber appeared to contribute less (R^2 = 0.59 – 0.77) to the array than the other 2 fibers. Based on changes in system coefficients that are part of a linear solvation energy relationship (LSER), these experiments were able to link physicochemical interactions in the MCF with those interactions in skin when either system is exposed to 1% SLS or 50% ethanol. These experiments further demonstrated the utility of a MCF array to adequately predict changes in dermal permeability when skin is exposed to mixtures containing either a surfactant or solvent, and provide insight into the nature of the physiochemical interactions which modulate dermal absorptions. (Supported by NIOSH OH-07555 and NIOSH OH 03669)

1557 SOLVATOCHROMATIC INTERACTIONS INFLUENCING DERMAL PERMEATION OF BIOCIDES IN COMMERCIAL CUTTING FLUIDS.

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Linear salvation energy relationships (LSER) can be used to explain the intermolecular interactions of the solute between 2 phases and to predict the dermal permeability of solutes. The aim of this study is to determine the interactions occurring

during dermal permeation of 4 phenolic biocides in 4 different formulations using a LSER approach, with a calibrated flow through diffusion cell system. Infinite doses of 4 biocides and 25 probe chemicals in water, 17% methanol and 2 commercial removal fluids namely Astrocut–C® and Tapfree 2® were applied to porcine skin flow through diffusion cells. The strength coefficients for the 25 probe compounds for each system were determined from multiple linear regression analysis and plugged into the Abraham's LSER equation to predict permeability values for biocides. Biocide permeability significantly decreased in methanol, Astrocut–C® and Tapfree 2® when compared to water. The strength coefficients revealed that hydrophobicity played an important role in explaining the reduced permeability in vehicles when compared to water. This finding is important for selection of biocides and cutting fluid formulations. The R^2 between experimental and predicted log Kp of probe solutes for water, methanol, Astrocut–C® and Tapfree 2® were 0.70, 0.78, 0.89 and 0.84 respectively. In conclusion, the LSER approach in part explained several chemical interactions resulting in reduced permeability and also predicted the dermal permeability of 4 biocides in commercial cutting fluids. Further dermal permeability of biocides will be determined in individual and combinations of different components of commercial cutting fluids to ascertain which component has prominent influence in dermal permeability. (Supported by NIOSH OH 03669)

1558 *IN VITRO* STUDIES OF PERCUTANEOUS ABSORPTION AND SURFACE-TO-SKIN TRANSFER OF D-METHAMPHETAMINE HYDROCHLORIDE USING HUMAN SKIN.

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Studies were designed to evaluate the percutaneous absorption and surface-to-skin transfer efficiency of [14C] d-methamphetamine hydrochloride (d-meth HCl) from contaminated materials to human cadaver skin, in vitro, using the finite dose technique and flow through diffusion cells.

The studies were performed on a minimum of three sections from three different cadaver skin donors. Two materials, vinyl tile and fabric, were used to measure the efficiency of d-meth HCl transfer from the material surface to skin. At pre-selected times during material-skin contact, the treated vinyl tile or fabric disks were removed from the upper surface of the skin, and skin incubation and receptor fluid collection were continued for up to 24 hours. In addition, the effect of wetting the fabric was evaluated to examine the potential influence of moist environmental conditions on transfer efficiency and skin absorption. All samples were analyzed for recovery of radioactivity using a liquid scintillation analyzer.

The results showed that d-meth HCl is very sensitive to changes in environmental pH. When the pH was greater than 4, the hydrochloride salt converted to its free base form, thereby increasing its volatility. d-Meth HCl penetrated into and through human cadaver skin quickly when skin was exposed to contaminated materials; transfer efficiency was directly related to contact duration. Moist conditions during the material surface-skin transfer accelerated transfer efficiency and subsequent percutaneous absorption. The smoothness and porosity of the contact surface also altered the efficiency of transfer. d-Meth HCl retained in the skin layer continued to be released into the receptor fluid even after the contact material had been removed. For future study, we recommend exploring the skin retention/penetration and skin surface decontamination/penetration relationships of d-meth HCl.

1559 COMPARATIVE EFFECTS OF SURFACTANTS (SLS AND LAS) ON THE DERMAL ABSORPTION OF A SERIES OF COMPOUNDS IN ISOLATED PERFUSED SKIN.

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Surfactants are a common constituent of many complex chemical mixtures encountered in occupational and environmental waste exposure scenarios. This study focused on assessing the effect of the surfactants sodium lauryl sulfate (SLS) or linear alkylbenzene sulfonate (LAS) on the dermal penetration and absorption on aqueous solutions of six chemicals (fenthion, parathion, ρ-nitrophenol, phenol, propazine and triazine) dosed topically on the isolated perfused porcine skin flap (IPPSF) model. SLS and LAS were selected because of their widespread applicability to practical dermal exposure scenarios, and their molecular similarity differing only in the absence or presence, respectively, of an aromatic ring in their polar head region. IPPSF absorption was assessed into perfusate, stratum corneum, and skin using radioassay of ^{14}C labeled compounds, with absorption parameters for individually-dosed compounds in SLS and LAS compared to aqueous controls without surfactant. Across all penetrants, absorptive flux and skin penetration was greater with SLS compared to LAS. The relationship of absorption from water varied, with surfactant treatments less than water for ρ-nitrophenol, parathion, fenthion and propazine, while absorption from water was intermediate between SLS and LAS for phenol and triazine. Surfactants also had variable effects of maximum flux. This work begins to establish a relationship between the physical chemical properties of penetrants and properties of the surfactant, an important phenomenon to understand when predicting chemical absorption from common mixtures. (Supported by NIOSH OH-07555)

1560 DERMAL EXPOSURE INDUCES KERATIN ADDUCTS IN THE SKIN.

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Polyaromatic hydrocarbons such as naphthalene are ubiquitous in the environment. Significant potential exists for naphthalene toxicity through dermal and inhalation exposure. Dosimetry and possible mechanisms of action through contact with respiratory epithelium and skin are poorly understood. Using 3-D reconstructed human skin exposed to naphthalene we observed that epoxide metabolites of naphthalene are generated by specific cytochrome P450 isoforms (e.g., CYP 2E1) in viable epidermis and form adducts with skin proteins such as keratin 1 (K1) and keratin 10 (K10). We then developed and tested enzyme-linked immunosorbent assays (ELISA) for dermal samples collected by tape stripping from workers exposed to naphthalene-containing jet fuel to quantitate naphthyl-keratin adducts as biomarkers of exposure to naphthalene. The four naphthyl-keratin adducts were detected at 0.170 ± 0.159, 0.127 ± 0.120, 0.495 ± 0.342, and 1.852 ± 1.067 pmole adduct/μg keratin for 1-naphthyl-K1, 2-naphthyl-K1, 1-naphthyl-K10, and 2-naphthyl-K10, respectively. Quantitation of keratin adducts obtained from the stratum corneum of exposed individuals will allow us to investigate the importance of dermal penetration, metabolism, and adduction of keratin as well as to make accurate prediction of the contribution of dermal exposure to the systemic dose for inclusion in exposure- and risk-assessment models.

1561 EFFECT OF BLOCKING THE IL-1 RECEPTOR ON JP-8-INDUCED GENE EXPRESSION IN RAT SKIN.

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Jet Propulsion fuel-8 (JP-8) may cause inflammation as a result of skin contact. Interleukin-1 alpha (IL-1 alpha), a proinflammatory cytokine, is preformed and stored in the epidermis. We hypothesize that the release of the preformed IL-1 alpha plays an important role in the inflammatory cascade induced by JP-8. To test this hypothesis, we applied JP-8 on rat skin after blocking the IL-1 alpha receptor using the IL-1 receptor antagonist (IL-1 ra) (Kineret, Amgen, Thousand Oaks, CA) and studied the changes in gene expression. After anesthetizing the rats, 100 micro liters of IL-1 ra or phosphate buffered saline (control), was injected into the skin without disrupting the barrier layer at the region of exposure. One hour after the injection, 500 micro liters of JP-8, in a gauze-filled, occlusive, plastic patch (3.14 cm2), was applied to the clipped back of male Fischer 344 rats for one hour. The rats were humanely killed using CO2. Injected and exposed skin was removed, placed in ice-cold RNAlater (Ambion, TX), and stored at 20 ○C overnight. Total RNA was isolated from the skin and labeled using Affy Target Labeling Kit. Biotinylated RNA was hybridized to rat genome 230 2.0 GeneChip arrays (Affymetrix) containing 31,099 genes. The chips were then scanned and the digitalized image data was processed using GCOS software (Affymetrix) and analyzed with GeneSpring GX 7.3 (Agilent Technologies, CA). The analysis showed two-fold changes in only 170 genes out of 31,099 probe sets. Up-regulated genes downstream of IL-1 were Lef1, Wnt 5a, and orosomucoid 1. The change in gene expression with IL-1 blockade suggests that JP-8 irritation may involve the release of preformed IL-1 alpha. (Sponsored by AF Office of Scientific Research)

1562 COMPARISON OF GENE EXPRESSION IN THE EPIDERMIS AFTER BRIEF EXPOSURES TO JP-8 IN HUMAN VOLUNTEERS AND RATS.

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Various groups have studied the irritation of the jet fuel, JP-8, in a wide variety models and have shown that inflammatory and growth pathways are activated by JP-8. We have investigated the time course of gene expression in rat epidermis after 1 h exposures to JP-8 (Tox Sci, 95(2): 495, 2007). The purpose of this investigation was to determine if an identical exposure to the skin of human volunteers would re-

sult in similar changes in gene expression. Twelve adult male volunteers (ages 18 to 57) were divided into 3 groups (1, 4 & 8 h) and exposed to 0.5 ml JP-8 and sham treatment. After 1 h, the chambers were removed and the skin was wiped with gauze. 8 mm punch biopsy were obtained under local anesthesis from JP-8- and sham-exposed sites, 1, 4 and 8 h after the beginning of the exposure. The epidermis was separated from the rest of the skin with a cryotome and total mRNA was isolated. Gene expression was measured with standard Affymetrix microarray (HG-U133 plus 2) techniques and changes from sham treatment were analyzed with GeneSpring GX 7.3 (GS) and Ingenuity Pathway Analysis (IPA). Variability in gene expression between individuals was large; therefore each individual was used as his own control. According to GS there were 51 genes changed ($p < 0.05$) in all subjects at 1 h; 103 changed at 4 h; and 45 changed at 8 h. The human results were in contrast to the rat results, where the largest number of changes was at 8 h. Serotonin signaling pathways were activated at each time point. Alanine/aspartate, ubiquinone, and retinol metabolic pathways were activated at each time point after exposure. Interestingly, the genes that were expressed in these pathways were not identical for each individual, reflecting more variability in the human population than the rat population. We conclude that the human and rat gene expression responses to JP-8 are similar, although the time of maximum response appears to be earlier in humans, and individual human variability complicates the analysis. (sponsored by AF Office of Scientific Research)

1563 GENE EXPRESSION ANALYSIS OF RAT EPIDERMIS AFTER 1 HOUR JP-8 AND JP-8 COMPONENT EXPOSURES BY REAL-TIME PCR.

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JP-8 jet fuel is a complex mixture of many aliphatic and aromatic hydrocarbons which can vary in composition from batch to batch. Occupational dermal exposures to jet fuels occur in personnel involved in aircraft refueling and maintenance operations and may cause irritation in these individuals. Changes in gene expression have been shown to occur in skin exposed to various individual components of the JP-8 mixture. Furthermore, these molecular changes occur before any visible signs of redness or irritation on the skin. In order to compare the early irritation response of jet fuel and some important components on the skin, we have chosen 30 different mRNA transcripts related to skin irritation. Total RNA was isolated from rat epidermis following a brief (1 hour) pure liquid exposure to either pure jet fuel (JP-8), dimethyl napthalene (DMN), undecane (UND), tetradecane (TET) or trimethyl benzene (TMB). Each transcript was assayed with the TaqMan real time quantitative PCR assay using TaqMan Gene Expression Assays. None of the components exactly mimicked the gene expression of JP-8, but UND had only three transcript responses that were different from JP-8. Nearly half (12) of the selected transcripts were either significantly upregulated or downregulated in all four components just like with JP-8, which indicates a similarity of responses. Chemokines (CCL4, CXCL1, CXCL2, and CCL20), cytokine receptors (TNF rec 1A, IL-6 rec and IL-1 rec I), growth-related transcripts (EGR1, MT3, PDGDS2 and RDC1) and the cytokine (IL-10) were changed in all treatments. This comparison suggests that the specific gene responses of all four hydrocarbon components were similar to JP-8, but none of the responses were identical. Of the 4 components tested with one-hour exposures, UND had the most similar gene expression response to JP-8. (Sponsored by the AF Office of Scientific Research)

1564 THE SECOND STAGE OF THE TOXICOGENOMICS PROJECT IN JAPAN: A MULTICENTER VALIDATION STUDY OF GENE EXPRESSION IN RAT LIVER.

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Transcriptome analysis is expected to be a useful tool for evaluation of drug safety in drug development. We performed our first 5-year collaborative study in the Toxicogenomics Project by the government and pharmaceutical companies in 2002 in which rats were exposed to chemicals (mainly medicines) and gene expression in liver (kidney in some cases) was measured by Affimetrix GeneChip and collected together with classical toxicological data. Experiments were also done with rat and human hepatocytes and more than 8 hundred million gene expressions for more than 150 chemicals were obtained by 2007. The data were combined with analysis and prediction systems established under the name of TG-GATEs (Genomics Assisted Toxicity Evaluation System). In order to utilize this system effectively, the second stage of the Toxicogenomics Project was started in 2007. When transcriptome data are analyzed, the inter-laboratory variations are problematic. In the first step of the project, we considered that a multicenter validation study was inevitable to use the system in the member companies. Acetaminophen was selected for this purpose and 300 (sub-toxic) or 1000 (toxic) mg/kg was administered to rats and gene expression in liver was measured by GeneChip 24 hr after dosing (N=5). Eleven out of 13 pharmaceutical companies joined in the project are enrolled in this study. At the beginning, comparison of SOP of each company revealed that various minor differences were found. At present, measurements and analyses are in progress. We expect to detect factors affecting data comparison and exchangeability among the laboratories and especially the reproducibility of detection of the drug-response. This work would supply essential and basic data for regulatory science in the near future.

1565 ONE-YEAR ASSESSMENT OF AFFYMETRIX RAE230 ARRAY PERFORMANCE USING MIXED TISSUE RNA REFERENCE SAMPLES.

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Microarray assays play an instrumental role in biological studies and toxicogenomics in particular. In many contexts, libraries of samples and expression data are generated as references for classifying a sample in question. Within the drug discovery and investigative toxicology context, such libraries are used to help classify and predict potential toxicities of a new compound based on expression data of known toxicants. Success of this approach is dependent on the reliability and reproducibility of sample preparation and the chosen array technology. Two rat-tissue mixtures (heart, liver, kidney and testis) have been previously introduced as RNA reference samples for defining target ratios on multiple microarray platforms. At Amgen, these samples are used to monitor data quality and process drift, thereby verifying the comparability of expression data obtained over time. This study presents results of the analysis of these two rat mixed tissue samples for the 12-month period starting July 2006. Intensity data were archived in the Rosetta Resolver system. The number of signature sequences and background noise did not change significantly over time. Correlations between chronologically consecutive profiles showed consistent intensity distributions. The receiver operating characteristic (ROC) plots showed sensitivity and specificity consistent with arrays previously classified as well performed. Target ratios between selected tissue-specific probes (100 per tissue) were well separated between testis and kidney tissues and, as expected, not as well distinguishable for liver and brain samples.

1566 ANALYSIS OF GLOBAL PATTERNS IN MICROARRAY GENE EXPRESSION DUE TO LOW DOSE ARSENIC EXPOSURE USING CORRELATION AND GENE SET ENRICHMENT.

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Gene microarrays successfully elucidate potential mechanisms associated with low dose arsenic toxicity, revealing diverse pathway targets such as immune response, oxidative stress, cell signaling, lipid metabolism and endocrine disruption that confirm other experimental results on a more global scale or lead to new hypotheses regarding targets and mechanisms of action. While pathway concordance is reassuring, concordance among short lists of the most significantly regulated genes from different arsenic experiments has, to date, been relatively rare, making general conclusions about arsenic effects difficult. Though one can attribute lack of agreement at the gene level to obvious differences between species and tissues, and it is widely accepted that arsenic has multiple biochemical and biophysical effects, one still expects experiments performed in different ways to show patterns of agreement beyond what would be expected by chance. We have investigated the concordance of different arsenic microarray results using two intuitive approaches to look for global patterns: 1) correlation in the gene regulation of gene homologs and 2) gene set enrichment in pathways that show consistency between multiple experiments. Early results based on in vivo chronic 10 ppb mouse lung arrays, and equitoxic chronic levels of arsenic in two human cell lines produce concordant results using these approaches. In particular, cell signaling and lipid metabolism pathways were consistently altered by comparable arsenic treatments in these different experiments and models. Such concordance is consistent with the hypothesis that low dose arsenic alters gene expression in predictable ways, based on underlying shared mechanisms, allowing us to make new testable hypotheses about potential molecular targets that serve as the basis for these effects.

1567 PROBABILITY FOLD CHANGE RANKING: A NOVEL COMPUTATIONAL ALGORITHM FOR IDENTIFYING ROBUST DIFFERENTIALLY EXPRESSED GENE LISTS.

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Identifying genes that are differentially expressed under different experimental conditions is a fundamental task in microarray gene expression studies. Currently, there are a few computational algorithms used for the identification of differentially expressed gene lists in microarray gene expression profiling studies. However, different ranking algorithms generate very different differentially expressed gene lists, which make the follow-up analyses and biological interpretation very difficult. Therefore, the development of a better gene ranking and selecting algorithm is critical in the microarray data analysis. We developed a new gene ranking algorithm, the probabilistic fold change (PFC) which ranks genes based on a confidence interval estimate of fold change, for identifying differentially expressed gene list. The performance of PFC ranking algorithm was tested using the MicroArray Quality Control (MAQC) data including rat toxicogenomics and qRT-PCR data sets as well as Latin square spike-in data sets. PFC ranking algorithm was found to outperform other popular gene ranking algorithms such as Mean Fold Change, SAM, t-statistic, Bayesian-t, Intensity-conditioned Fold Change for the identification of differentially expressed gene lists. Of all gene ranking algorithms tested, PFC not only produced better reproducible gene lists but also offered better sensitivity in identifying differentially expressed gene list. The findings of our analyses may provide some insight toward better data analysis approach in identifying reproducible differentially expressed gene lists derived from microarray toxicogenomics study to help make sense of the data with better biological interpretation.

1568 CHEMICAL TOXICITY PREDICTION FOR TOXCAST PHASE I.

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U.S. Environmental Protection Agency is undertaking the ToxCast program to develop cost-effective innovative approaches to prioritize a large number of chemicals for further toxicological testing. In Phase I, ToxCast uses genomic data and high-throughput screening data to create predictive bioactivity signatures, based on the known toxicity of 300 chemicals. We propose a method for toxicity prediction based on gene expression profiles, combining microarray data analysis with machine learning approaches. Each step of our method involves standard approaches, while collectively providing robust and effective prioritization.
1. We apply SAM, Limma or other appropriate methods to detect significantly differentially expressed or dose-responsive genes. These methods apply penalties to lowly expressed genes and provide false-discovery rate error control. 2. Among the genes declared significantly differentially expressed, we identify those that appear for at least k chemicals. As a further regularization approach, we create a binary dataset by recording whether or not the gene is significantly differentially expressed. 3. We randomly split this binary dataset into a training set and test set, performing feature selection and prediction on the training set using several machine learning methods (support vector machine, random forest etc.) The test set is then used to evaluate the classifier performance. 4. We evaluate the performance of different competing classifiers and identify the optimal prediction method.
Our overall approach combines sophisticated microarray data analysis with various machine learning methods. One advantage is that our use of binary derived data reduces the dependence on the specific method of significance testing and array platform, and thus may provide ready extension to later phases of ToxCast program. In addition, the binary structure simplifies the process of feature selection through combination of data into a signature gene index. The approach is illustrated using analysis of MAQC (MicroArray Quality Control Project) datasets.

1569 GENE EXPRESSION PROFILING IN RAT LIVER TREATED WITH COMPOUNDS INDUCING PHOSPHOLIPIDOSIS.

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We have constructed a large-scale transcriptome database of rat liver treated with various drugs. In an effort to identify a biomarker for diagnosis of hepatic phospholipidosis, we extracted 78 probe sets of rat hepatic genes from data of 5 drugs, amiodarone, amitriptyline, clomipramine, imipramine, and ketoconazole, which actually induced this phenotype. Principal component analysis (PCA) using these probes clearly separated dose- and time-dependent clusters of treated groups from their controls. Moreover, 6 drugs (chloramphenicol, chlorpromazine, gentamicin, perhexiline, promethazine, and tamoxifen), which were reported to cause phospholipidosis but judged as negative by histopathological examination, were designated as positive by PCA using these probe sets. Eight drugs (carbon tetrachloride, coumarin, tetracycline, metformin, hydroxyzine, diltiazem, 2-bromoethylamine, and ethionamide), which showed phospholipidosis-like vacuolar formation in the histopathology, could be distinguished from the typical drugs causing phospholipidosis. Moreover, the possible induction of phospholipidosis was predictable by the expression of these genes 24 hr after single administration in some of the drugs. We conclude that these identified 78 probe sets could be useful for diagnosis of phospholipidosis, and that toxicogenomics would be a promising approach for prediction of this type of toxicity.

1570 GENE EXPRESSION PROFILING IN TOXICOLOGICAL STUDIES USING FFPE TISSUES.

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Large numbers of FFPE tissues from toxicology studies have been accumulated. Although RNA extracted from FFPE tissue is significantly degraded, new methods are allowing their use for gene expression analysis.
In this study, the DASL (cDNA-mediated annealing, selection, extension, ligation) Assay from Illumina and the WT-Ovation™ FFPE-System from NuGEN were used. Both methods, compared to standard technologies, do not need intact mRNA poly-A tails for priming. The major aim was to compare these platforms and simultaneously evaluate the effect of formalin fixation time and RNA extraction techniques.
Livers from 3 untreated rats were fixed for different times. Using RNA extracted with either High Pure RNA Paraffin (Roche) or RNeasy® FFPE Kits (Qiagen), the DASL Assay was performed to reverse transcribe, amplify, label and hybridize samples onto a custom DASL-BeadChip (512 liver toxicity genes). A subset of samples was processed using the NuGEN system and hybridized onto Affymetrix Rat 230 2.0 GeneChips®.
The best correlation of gene expression was found between samples extracted with the High Pure RNA Paraffin Kit. Longer fixation times resulted in increased alterations of signal intensities correlating to increased degradation of mRNA. Fresh frozen samples compared to 1 day, 1, 2, 4 weeks fixation for DASL showed changed intensities for 74, 105, 152, 157 genes, respectively. More than 10,000 genes showed changed signal intensities for fixed samples compared to fresh frozen samples with NuGEN and rising by fixation time. The number of present calls on Affymetrix decreased by longer fixation and after 4 weeks the present calls were in the range of background. Overall, correlation coefficients dropped by increased fixation times in both platforms.
In conclusion, results of gene expression analysis are heavily dependent on RNA extraction and fixation times. This study showed that ideally tissues should not be fixed for longer than 1 day to obtain good gene expression data. Both techniques provided comparable results and are appropriate methods for gene expression analysis using FFPE tissues.

1571 HEPATIC GENE EXPRESSION DURING AGING IN MALE AND FEMALE MICE.

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The incidence of liver cancer increases during aging. The incidence of liver cancer is higher in male than female. In order to explore the possible mechanisms that account for these differences, we compared the gene expression profiles of livers obtained from 24-month (aged) and 6-month (mature) old male and female mice using microarray technology (GeneChip® Mouse Genome 430A 2.0 Array). Each gender and age group has three mice. The threshold for a significant change was set greater than 2 fold. Cancer related genes are classified into sub-groups according to their roles in carcinogenesis and disease process. In the male aged mice group, many pro-apoptosis and pro-metastasis genes were up-regulated while anti-apoptosis and anti-metastasis genes were down-regulated. Genes involved in promoting or inhibiting cell cycle transition and associated with DNA repair such as Myc (17-fold), Fos (7-fold), EGR1 (Early Growth Response 1, 13-fold), and HSPA1A (Heat Shock 70K Da Protein 1A, 44-fold) were found to be expressed at a reduced level in aged male mouse livers compared with the matured male mouse livers. In female aged mice, the most notably change in hepatic gene expression was found in genes that are related to immune system. For example, the expression of the CD74 (HLA-DR-gamma, 3-fold) and C1QA (complement component 1, q subcomponent, 4-fold) genes were up-regulated. In contrast to male mouse livers, no clear change trend was noted in other cancer related genes in aging female mouse livers. In conclusion, the hepatic gene expression profile changes during aging. Those changes might increase the susceptibility of aged mice to liver cancer. In addition, more changes occurred in male than female aged livers. Such difference might account for gender difference in the incidence of liver cancer. (NIH R01 CA053596).

1572 AGE-RELATED HEPATIC GENE EXPRESSION ALTERATIONS IN IMMUNE SYSTEM PATHWAYS IN THE FISCHER RAT.

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Aging is associated with alterations in multiple components of the immune response, affecting both cellular and humoral immunity. Evaluation of immune system alterations is a key element in long-term toxicity and carcinogenicity studies. Understanding age-related changes in transcript levels of immune function genes is critical to accurate interpretation of data from such studies. Therefore, we examined differential expression of immune-related genes in the liver of untreated male F344/N rats at 32, 58 and 84 weeks of age to elucidate potential dysfunction in the immune response that may occur solely as a result of age. RNA was analyzed using the Agilent Rat Oligo Microarray and Statistical Analysis of Microarray (SAM). Approximately 288 genes associated with immune function were differentially expressed in 84 week old animals relative to 32 week old animals. The most significantly and uniquely affected pathway was the complement and coagulation pathway, with both the classical and alternate pathways affected. Several cytokine and growth factor pathways were altered, specifically interferon (IFN), interleukin (IL)-2, IL-4, IL-6 and granulocyte macrophage colony stimulating factor (GM-CSF). A series of related Janus kinases (JAKs) and Signal Transducers and Activators of Transcription (STAT) molecules transmit signals from receptor to cell nucleus, where gene expression may be modified, and the changes in the JAK/STAT pathway were a common feature among the cytokine and growth factor pathways. Marked alterations were also observed in genes associated with inflammation and the inflammatory response, T-cell function, B-cell receptor signaling, and apoptosis. These findings are consistent with published phenotypic alterations in immune parameters. The results of this study indicate that immune competence of male rats changes as the animals approach twenty months, and that awareness of these changes is important when interpreting the results of long-term studies.

1573 COMPARATIVE HEPATIC GENE EXPRESSION ELICITED BY o, p'-DDT IN THE RAT AND MOUSE.

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Our previous study suggested that o,p'-dichlorodiphenyltrichloroethane (o,p'-DDT) elicits PXR/CAR, not estrogen receptor, mediated gene expression activity in the immature ovariectomized Sprague Dawley rat liver. In order to assess the conservation of o,p'-DDT's effects, we investigated expression profiles of several genes using a quantitative RT-PCR method in immature, ovariectomised C57BL/6 mice. Mice were gavaged with 300 mg/kg o,p'-DDT either once or once daily for three consecutive days. Liver samples were collected at 2, 4, 8, 12, 18 or 24 h after a single dose or 72 h following three daily doses. Drug metabolism enzyme (DME) genes (Cyp2b2, Cyp3a2 and Cyp3a23/3a13 in rats; Cyp2b10 and Cyp3a11 in mice) were induced in both species in a comparable manner, suggesting PXR/CAR activation. Induction of Gclm was also concerved between rats and mice, suggesting the perturbation of glutathione homeostasis associated with oxidative stress in response to DME induction. PXR induction and down-regulation of the lipid metabolism-associated gene, Srebf1, was also conserved in rats and mice. However, CAR was down-regulated in mice but induced in the rat at 8 h. Thus, although PXR/CAR activation by o,p'-DDT appears to be conserved, the regulation of CAR expression is different. Furthermore, steroidogenesis-related gene Cyp17a1 was induced in mouse liver (~8-fold) but not in rat. Consequently, the hepatic induction of Cyp17a1 in mice raises the possibility of enhanced steroidogenesis in mice compared to rats which might alter the estrogenic activity following o,p'-DDT exposure. Funding for this study was provided by NIGMS grant GM075838 and the US Environmental Protection Agency (EPA) grant RD83184701. TRZ is partially supported by the Michigan Agricultural Experiment Station.

1574 EARLY MOLECULAR MECHANISMS ASSOCIATED WITH HEPATOCARCINOGENESIS IN F344 RATS TREATED WITH GENOTOXIC, NONGENOTOXIC OR NONCARCINOGENS.

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The early identification of the potential for chemical carcinogenesis would be aided by the definition of the molecular pathways involved. Genotoxic compounds (GC) initiate tumour genesis by DNA damage causing mutations, whereas nongenotoxic carcinogens (NGC) perturb normal controls to form a permissive milieu for uninhibited proliferation of initiated cells. Using Affymetrix GeneChip, metabonomic biochemical and histological analyses, we investigated the alterations induced in rats that had been exposed to the NGCs phenobarbital (PB) chlorendic acid (CA) diethylhexyl phthalate (DP) monuron (MN) methapyrilene (MP); the non-mutagenic non-hepatocarcinogens (NHC) cinnamyl anthranilate (CN) diethylhexyl adipate (DA) benzophenone (BP) and diethylthiourea (DU); and the GC 2-acetyl aminofluorene (2AAF) in their diets for 7, 28 or 90 days. Adjusted liver weights increased at each time examined, except for CA and MP. Hepatocyte hypertrophy was seen at all times with DP, DA, CN, PB and BP. MP increased mitosis and necrosis. MP and CA increased plasma glutamate dehydrogenase activity. 5818 probe sets were statistically significantly altered ($p<0.05$ >1.5 fold vs. control) for at least one treatment and time. Gene Ontology and pathway analysis identified many changes associated with lipid or fatty acid metabolism. Both the NGCs and NHCs decreased plasma triacylglycerol, transiently for the latter. Similarities in gene expression were identified by Euclidean Distance and several clusters were apparent: (i) DA, DP, CN and 28 day BP (ii) PB and BP (iii) MP, CA and (iv) DU and MN. 2AAF was most similar to MP and CA but also had genes associated with DNA-damage differentially expressed. Combining these data with known regulatory information and our metabonomics analysis has provided greater insight into the different mechanisms utilised by these compounds.

1575 THE UTILITY OF TOXICOGENOMICS IN THE PRIORITIZATION OF 5-HT6 RECEPTOR ANTAGONISTS.

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Several inhibitors of serotonin (5-hydroxytryptamine) receptor 5-HT6 are currently being developed for treatment of disorders in the central nervous system. Based on previous internal observations, concerns about hepatotoxicity have been raised about this class of compounds. Two candidates, RO-X and RO-Y, were assessed in two-week, in vivo rat and cynomolgus monkey studies for safety. In both rats and monkeys, RO-Y treatment resulted in increased liver weights. Prior to completion of histological analysis, toxicogenomics on liver from both species was conducted to glean insight into the mechanism of hepatomegaly. RO-Y treatment revealed evidence of PXR and CAR-mediated enzyme induction, including significant perturbations in genes responsible for Phase I (Cyp2b, Cyp3a, Cyp1a), II (Ces2, Sult2a, Gst), III (Oat, Slc6a6, Abcc3), and cholesterol metabolism. A total of 58 genes were considered both biologically and statistically significant to the histological finding of RO-Y; however, these genes were not changed in response to RO-X treatment. Employing short term in vivo studies, these 58 genes were used in a multivariate, weight-of-evidence approach to prioritize subsequent 5-HT6 receptor antagonists. The toxicogenomics strategy described herein has provided a short term screening tool for potential induction and/or cholestatic hepatotoxicity in subsequent molecules.

1576 DETECTION OF UNIQUE TOXIC RESPONSES TO CARBON TETRACHLORIDE BY COMPREHENSIVE GENE EXPRESSION ANALYSIS USING CYNOMOLGUS MONKEYS AND RATS.

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Toxicogenomic analysis as part of the drug discovery decision-making process and the prediction of toxicity at an early stage of drug development are widely conducted. Compared with gene expression analysis in rats, there have been few analyses using the cynomolgus monkey. Cynomolgus monkeys are the most frequently used non human-primates for pre-clinical studies. In collaboration with Takeda Pharmaceutical Company, we produced a custom DNA microarray for this species using biopsied liver samples previously (45th SOT, 2006). In the present study, we studied differences in hepatotoxic molecular mechanisms between cynomolgus monkeys and rats by comprehensive gene expression analysis.

A typical hepatotoxicant, carbon tetrachloride (CCl4) was orally administered to 3 male cynomolgus monkeys and 5 male rats in a single dose of 1mL/kg. At 6 and 24 hours after administration, the livers were collected for gene expression analysis and histopathology. The expression profiles and the conventional toxicological parameters were compared between cynomolgus monkeys and rats.

The common response genes were categorized as up-regulation (calcium homeostasis and endoplasmic reticulum stress related genes) or down-regulation (phaseI drug metabolizing enzyme and apolipoprotein related genes) in CCl4 treatment, while oxidative stress and inflammatory related genes showed different response between cynomolgus monkeys and rats. In particular, inflammation related genes were associated time-dependently with histopathological findings in the liver (mononuclear cell infiltration).

From these results, it was concluded that gene expression profiles obtained from both cynomolgus monkeys and rats allow to detect the possible unique toxic response. In terms of extrapolation to humans, gene expression analysis of cynomolgus monkeys suggested benefits for analysis of molecular mechanisms of adverse effects in pre-clinical studies.

1577 HEPATIC TOXICOGENOMIC PROFILING IN RATS AS A PREDICTOR OF IDIOSYNCRATIC DRUG-INDUCED LIVER INJURY IN HUMANS.

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Idiosyncratic drug-induced liver injury (referred to here as DILI-2) is among the leading causes of acute liver failure in the United States. The mechanisms by which drugs cause idiosyncratic hepatotoxicity in humans are not well understood and these compounds generally fail to display classic signs of hepatotoxicity in animal studies. We tested the hypothesis that DILI-2 compounds induce a gene expression pattern in rats that is predictive for idiosyncratic hepatotoxicity in humans. Male rats were administered (iv or po) a single dose of DILI-2 compounds (Nefazodone, Nimesulide, Ranitidine, Sulindac, Tacrine, Tolcapone, Trovafloxacin), known hepatotoxicant (CCl4) or non-hepatotoxicants (Buspirone, Rosiglitazone) at doses that approached the maximum tolerated dose. Twenty-four hours after administration animals were euthanized and blood was collected for AST, ALT, and GLDH with liver samples collected for toxicogenomic analysis and histological evaluation. With the exception of CCl4, compounds did not induce significant changes in serum biomarkers of liver injury, nor did they result in histological evidence of liver damage. The toxicogenomic profiles of the livers were evaluated with predictive models from Gene Logic (Gaithersburg, MD) and for 5 of 7 DILI-2 compounds (Nefazodone, Nimesulide, Sulindac, Tacrine, Tolcapone) the gene expression signatures were found to be similar to those of compounds known to cause hepatitis, inflammation and fibrosis in humans. This work suggests that toxicogenomic analysis of liver tissue may be a sensitive tool in animals to identify compounds that have a potential risk for DILI-2 in humans.

1578 TOXICOGENOMIC COMPARISON OF RAT LIVER AND PRIMARY HEPATOCYTES FOLLOWING COMPOUND EXPOSURE.

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Hepatotoxicity is a major cause of compound attrition in the drug discovery and development process. It is thus desirable to obtain information on a compound's safety profile early on during this process. Primary hepatocytes treated *in vitro* have been proposed as a surrogate system for detection and understanding of drug induced hepatotoxicity much earlier in the discovery process. The goal of the present study was to compare the global gene expression patterns of rat liver and primary hepatocytes in response to treatment with common compounds. Recognizing that there will be differences in individual gene expression patterns between *in vivo* and *in vitro* hepatocytes, we chose to analyze the impact of treatment on gene expression across pathways. Sixty-three compounds of diverse structure activity classes were profiled in liver from treated rats and in primary rat hepatocytes. Hierarchical clustering across pathway impact scores for 110 diverse pathways revealed significant global similarities between liver and primary hepatocytes. Compounds of varied structure activity classes, many of which are hepatotoxicants, impact similar pathways in both liver and primary hepatocytes. Similarly impacted pathways include xenobiotic metabolism, glutathione regulation, and metabolic pathways. Compounds were then divided into groups based on their known hepatic liabilities, such as DNA damage induction, PPARα induction, GSH depletion, or induction of cholestasis. We show that many of the top impacted pathways are conserved between liver and hepatocytes for these hepatotoxicant sub-groups. These data demonstrate that at the pathway level, rat primary hepatocytes share many similarities with *in vivo* liver following exposure to diverse compounds. These findings provide additional rationale for building a hepatocyte toxicogenomics database to predict liver toxicity *in vivo*, and to aid in understanding mechanisms of action/toxicity.

1579 EXPLORING CYTOLETHALITY RESPONSE OF COMPOUNDS IN PRIMARY RAT HEPATOCYTES USING GENE EXPRESSION PATHWAY ANALYSIS.

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The primary rat hepatocyte in vitro model has been used extensively in toxicology over the last twenty years for understanding mechanism(s) of toxicity and screening compounds in drug development. We describe the use of the cytolethality assay, as judged by LDH leakage and gene expression on Affymetrix RG230v2 microarrays, to assess intrinsic toxicity and rank order compounds prior to in vivo testing. Hepatocytes were treated with 164 compounds that were categorized as either cytolethal (<=30μM LC20, n=102) or non-cytolethal (>=80μM LC20, n=62) for 24 hours. Compounds used in this study were selected from over 20 projects/targets with differing modes of action. By comparing pathways regulated for these two groups we determined specific and non-specific expression responses in this model system. These data show that potent cytotoxicity, as defined by an LC50 <30uM, can predict a >2-fold increase in serum alanine aminotransferase in vivo, with 87% sensitivity and 71% specificity. When we examined significantly up-regulated transcripts specific for cytotoxic compounds, 26 pathways were identified, including those for apoptosis, TGF beta, and WNT signaling. For non-cytolethal compounds, up-regulated transcripts encompassed 31 pathways that were distinct from those observed with cytolethal compounds. These included several inflammatory pathways, as well as pathways of mitochondrial and metabolic function. Specific transcripts and the 25 pathways regulated in common to both groups may be attributed to a non-specific cellular response not associated with cytolethality and therefore of less significance in predicting in vivo outcomes. Further analysis of these data will provide valuable insight into the response to drug treatment, and the biological attributes of this widely used toxicology model.

1580 GLOBAL TRANSCRIPTOME CHANGES IN HEPG2 CELLS EXPOSED TO COPPER.

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Copper is an essential trace element that has the potential to generate free radicals and oxidize cellular components through its redox activity. Our hypothesis is that copper modulates the activity of multiple intracellular signal transduction pathways to affect transcription. We have previously shown that copper activates transcription through both metal- and oxidative stress-responsive signal transduction pathways. Since the global molecular mechanisms underlying copper toxicity have not been well elucidated in humans, we have profiled transcriptome changes in HepG2 cells exposed to 100, 200, 400 and 600 μM copper for 4, 8, 12 and 24 hours using a human oligonucleotide microarray. Differentially expressed genes were identified, and integrated into biological and functional pathways through Gene Ontology analysis, which revealed that copper modulated genes associated with physiological adaptive responses such as transition metal ion binding and response to stress at lower concentrations (100 and 200 μM), and toxic responses such as apoptosis, morphogenesis, regulation of transcription, induction of signal transduction pathways, and negative regulation of biomolecule metabolism at higher concentrations (400 and 600 μM). Global gene expression profile was overlaid onto signal transduction cascades using pathway mapping, which demonstrated that copper could modulate additional signal transduction pathways such as death receptor, IGF-1, hypoxia, IL-10, IL-2, IL-6, EGF, Toll-like receptor, protein ubiquitination, xenobiotic metabolism, leukocyte extravasation, complement and coagulation, and Sonic Hedgehog signalings. We also performed interactome identification using biomolecular interaction networks, which revealed that HNF4α (hepatocyte nuclear factor 4 alpha) might be one of the major regulators to modulate the expressions of copper-responsive genes. All these results strongly supported our hypothesis, and allowed us insights into the global and molecular mechanisms of copper physiology and toxicity in HepG2 cells.

1581 ARSENIC PERTURBATION OF GENE NETWORKS DURING VERTEBRATE DEVELOPMENT.

A. Planchart and C. J. Mattingly. *Mount Desert Island Biological Laboratory, Salisbury Cove, ME.* Sponsor: W. Toscano.

Arsenic is a ubiquitous environmental toxicant that is associated with several human diseases, including hypertension, diabetes and cancer. The primary mode of ingestion in humans is via contaminated drinking water. Consequently, the U.S. EPA mandated that public water supplies deliver no more than 10 parts per billion (ppb) of arsenic (10 μg/L) under the assumption that this exposure level poses minimal risk. However, no studies have addressed the potential for currently acceptable levels of arsenic to perturb gene networks critical to vertebrate embryo development. To investigate if arsenic poses a significant risk during vertebrate embryogenesis, we measured gene expression changes in zebrafish embryos exposed to arsenic. AB wild-type zebrafish embryos were collected after spawning and exposed, in triplicate, to 0, 10 and 100 ppb aqueous arsenic (sodium meta-arsenite). After 48 hours, total RNA was extracted from embryos, converted to labeled cRNA and used to probe Affymetrix zebrafish genome arrays, one gene chip per replicate. The data were collected, normalized, log-transformed and subject to ANOVA to identify arsenic-induced gene expression changes.

We identified 91 genes (10 ppb) and 98 genes (100 ppb) that were differentially expressed in comparison to controls. Many genes were unique to each exposure with the exception of 10 genes perturbed under both concentrations. Of

these, eight were up-regulated (average of 1.77-fold, P ≦ 0.05) and two were down-regulated (average -1.66-fold, P ≦ 0.05) relative to controls. Gene regulatory network analysis revealed that four of the ten genes belong to a common signaling pathway that is critical to vertebrate development and neurological function.

Our analysis indicates that arsenic significantly perturbs gene regulatory networks important in embryonic development. Furthermore, a subset of development-critical genes are perturbed at doses deemed safe, suggesting that current exposure limits may pose significant risks to humans during development particularly if arsenic is capable of crossing the placental barrier.

1582 D-SERINE EXPOSURE RESULTED IN GENE EXPRESSION CHANGES IMPLICATED IN NEURODEGENERATIVE DISORDERS AND NEURONAL DYSFUNCTION.

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D-serine is involved in many physiological processes (e.g. development, learning, and cell-death signaling) through its interaction with the strychnine-insensitive glycine binding site of the N-methyl-D-aspartate receptor (NMDAR). Recent evidence suggested that decreased function of the NMDAR is related to the etiology of schizophrenia, and the use of D-serine as add-on pharmacotherapy is beneficial in alleviating the symptoms of treatment-refractory schizophrenia. As the NMDAR also plays a major role in neuronal cell death mediated by excitatory amino acid toxicity in ischemia, epilepsy, and trauma, D-serine can markedly potentiate NMDAR-mediated neurotoxicity. To investigate potential adverse effects of D-serine treatment, we investigated gene expression changes in the frontal lobe of the brain from male F-344 rats treated with a single dose of D-serine (5 - 500 mg/kg) at 96 hours post-treatment. Gene expression profiling using the Affymetrix RAE230A GeneChip arrays revealed that D-serine treatment resulted in up- and down-regulation of 135 and 51 genes, respectively, based on the combined results of t-test, ANOVA and Significance Analysis of Microarray. Gene ontology and pathway analyses revealed that genes involved in pathways implicated in neurodegenerative disorders (e.g. insulin, Notch, and Wnt signaling pathways, focal adhesion, and ubiquitin proteasome pathway) are over-represented in the final gene list. Also over-represented are the genes involved in biological processes essential for neuronal function (e.g. vesicle-mediated transport, endocytosis, and small GTPase mediated signal transduction). Taken together, these findings highlight the impacts of D-serine exposure on neuronal function in that perturbation of essential pathways persists even 4 days after the treatment.

1583 EFFECTS OF ETHANOL ON GENE EXPRESSION IN THE BRAIN.

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Recent advances in microarray technology are enabling toxicologists to study the effects of chemicals in organisms on molecular levels never before explored. Here we studied the effects of alcohol (EtOH) on gene expression in the brain after a period of EtOH intake. 19 mice consumed EtOH in a two-bottle choice paradigm, and daily intake was recorded. After 16 days of exposure and 6 days of washout, the mice were sacrificed, and tissue samples were collected from each of three brain regions: the nucleus accumbens (NAC), the prefrontal cortex (PFC) and the ventral tegmental area (VTA).

RNA was extracted from each sample and hybridized to an Affymetrix GeneChip. After image analysis, probe level data was stored in a *.CEL file for each brain region of each mouse. All subsequent quality control (QC) and statistical analyses were conducted using the R programming environment (v2.5.1) using the affy library and appropriate R and Bioconductor packages. After QC, all Affymetrix control probe sets were removed from the data set, and expression summaries were obtained using the Robust Multiarray Average (RMA) method.

It was of specific interest to determine which genes are univariately associated with EtOH intake. With 16 observation points for each of the 19 mice in the study, concern was raised for the potential of an over-fit model. Therefore, a principle components analysis was conducted to reduce the number of explanatory variables. While 5 principle components explained 91% of the variance present in the data (exceeding the recommended 90% level), the possibility of an over-fit might still be of concern. For this reason, models were built using both five and two principle components, as two principle components explained an acceptable 77% of the observed variation. Thereafter, gene-specific linear models predicting expression as a function of the independent additive principle components in each of the three brain regions were fit. Genes significant at 0.001 level were considered important, and biological implications of those genes were subsequently identified.

1584 GLOBAL GENE EXPRESSION ANALYSIS IN A COMMON MARMOSET MODEL OF HALOPERIDOL-INDUCED ACUTE DYSTONIA.

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Animal models are used for the prediction of drug-induced extrapyramidal symptoms, main side effects of neuroleptic agents. Traditionally, a rat catalepsy model was used for the purpose, but its predictivity was found to be poor. We have already established a haloperidol-induced acute dystonia model using the common marmoset (Callithrix jacchus), a non human primate, and found that the model showed a good correlation with human clinical results. In this study, we used an Affymetrix-based custom marmoset oligonucleotide microarray containing 7,512 probe sets for investigating gene expression profiles of the marmoset acute dystonia model. Marmosets were treated with haloperidol orally twice a week over 8 weeks until dystonic behavior was observed. Four hours after the final treatment, caudate nucleus, lentiform nucleus and ventral midbrain were isolated and subjected to DNA microarray analysis. Global analysis showed 384 genes were differentially regulated between normal animals and animals with dystonia. Most of those genes did not overlap with differentially regulated genes in animals with single haloperidol treatment. Lentiform nucleus produced the most drastic changes in gene expressions, compared with other brain regions. Several of differentially regulated genes were reported as affected genes in humans with Parkinson's disease or in dystonia models using other animal species. These results suggest that the genomic approach using the custom DNA microarray in combination with the marmoset model is valuable for studying the molecular mechanism of psychiatric disorders, neurological diseases and side effects of antipsychotic drugs.

1585 TRANSCRIPTOMIC ANALYSIS OF TOLUENE-EXPOSED BROWN NORWAY RAT BRAINS AT DIFFERENT LIFE STAGES.

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Differential susceptibility to environmental exposures in subsets of the population is a major regulatory concern of the Environmental Protection Agency. Of special interest is the elderly, the fastest growing segment of the population who may be considered a special at risk group because of physiological changes in sensitivity, elimination, homeostatic and/or repair mechanisms. In this study global genomic changes (Affymetrix rat 230A chips) were examined after acute oral toluene exposures (0, 650 and 1000 mg/kg) at 3 ages (4, 12 and 24 mos) in Brown Norway Rats (4 animals/gp; 36 arrays total). Hippocampi were dissected out 4 hrs after exposure, gene expression determined and data analyzed across age and dose groups to identify response to toluene and the effect age has on that response. Two-way ANOVA ($p < 0.05$) identified 1418 genes differentially expressed with age across any treatment group, 206 genes changed with dose, and 198 genes through an interaction between the two parameters indicating that the impact of age alone is much greater than the effect of acute toluene exposures. However, the data also show, as indicated by the number of genes displaying an age/dose interaction, that life stage can impact toxic response. Further analysis to identify the genes that differed between groups documented 595 genes that changed between 4 and 12 mos of age and 610 genes between 12 and 24 mos with 127 of those genes differing across all 3 age groups. Similar analysis of genes that changed with dose found 53 genes that were different between the 650 and 0 mg/kg toluene groups, 73 genes between the 1000 and 650 mg/kg doses and 10 genes that were in common across doses. Expression of genes with roles in immune response (up-regulated) or cytoskeleton, protein metabolism and energy metabolism (all down-regulated) were changed as life stage progressed, indicating changes in basic cell homeostasis. These data will help identify processes for further investigation into the role of aging in susceptibility. (This abstract does not necessarily reflect USEPA policy).

1586 EXPRESSION PROFILING OF FIVE RAT STRAINS REVEAL TRANSCRIPTIONAL MODES IN THE ANTIGEN PROCESSING PATHWAY.

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Gene expression profiling of rat strains with predisposition to cardiovascular diseases can help decode the transcriptional program that governs cellular behavior. We hypothesized that co-transcribed, intra-pathway, functionally coherent genes can be revealed from analysis of microarray data from ozone-exposed rat strains. Male 12-14 wks old Wistar Kyoto (WKY, healthy), Spontaneously Hypertensive

(SHR), Stroke-Prone SHR (SHRSP), Heart Failure SHR (SHHF) and Diabetic (JCR) rats were exposed to air or ozone (1ppm) for 4 hrs. At the conclusion of the exposure period, lung tissue was extracted and analyzed for gene expression using rat 230A Affymetrix GeneChips. Principal component analysis of the 10 experimental conditions demonstrated remarkable strain-related and ozone-treatment induced differences in gene expression. Kyoto Encyclopedia of Genes and Genomes pathway analysis identified from 9 to 22 pathways differentially expressed in these rat strains regardless of ozone ($p<0.05$ by hypergeometric test of over-represented differentially expressed genes). One of the pathways with more significantly altered genes, the antigen processing pathway of SHHF, was chosen for further analysis. For the thirty genes comprising this pathway a correlation matrix across the ten experimental conditions (both air and ozone combined) was computed. Amazingly there were only two groups of co-transcribed genes. The first group consisted primarily of MHC class II processing genes and the second group was comprised primarily of inflammation and acute phase response mediating genes. In addition the first group was upregulated in the strains with likely healthy immune systems, WKY and JCR; whereas, the second group was upregulated in strains with compromised immune systems, SHHF, SHR and SHRSP. Thus, the transcribed modules were related to the immune competence of the respective strains. We believe this analysis may reveal transcriptional modules with functionally cohesive genes that express unique disease phenotypes or toxic responses to environmental exposures. (This abstract does not reflect US EPA policy.)

1587 GENE EXPRESSION PROFILING OF NORMAL HUMAN BRONCHIAL EPITHELIAL CELLS EXPOSED TO TRIVALENT ARSENICALS AND DIMETHYLTHIOARSINIC ACID.

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Lung is a major target for arsenic carcinogenesis in humans. However, the carcinogenic mode of action of arsenicals is unknown. We investigated, in human bronchial epithelial (BEAS-2B) cells, the effects of inorganic arsenic (iAsIII), monomethylarsonous acid (MMAIII), dimethylarsinous acid (DMAIII) and dimethylthioarsinic acid (DMTA), a sulfur-containing dimethyl arsenic metabolite. Cells were exposed to 3, 15 μM-iAsIII; 0.3, 1 μM-MMAIII; 0.2, 1 μM-DMAIII; 0.2, 0.9 μM-DMTA as non-cytotoxic and minimally cytotoxic (~20%) concentrations based on neutral red uptake assays after 24 h of culture. Total RNA was isolated and gene expression analysis conducted using Affymetrix® Human Genome 133 Plus 2.0 arrays. Differentially expressed genes (DEG) were determined using a one-way ANOVA ($p\leq0.05$) by Rosetta Resolver®, a Benjamini-Hochberg FDR multiple testing correction (<0.05) and a $\pm$ 1.5 fold change cut-off. For all compounds except DMTA, >90% of DEG altered in the low concentration were also changed at the high concentration. iAsIII showed the highest number of DEG at both concentrations (2708 and 123, high and low, respectively). 1749, 420 and 120 DEG were unique to the high concentrations of iAsIII, MMAIII and DMAIII, respectively. Ingenuity Pathway Analysis™ revealed p53 signaling (E2F1& 2, SERPIN), and cell cycle related genes (cyclinD1) were altered by the high concentrations of DMTA, MMAIII and iAsIII. Oxidative stress (DUSP1, GPX2, NQO1) and NFκB signaling (TLR4, NFkB) pathways were changed in the high concentrations of MMAIII and iAsIII. Additional pathway analysis will better define the biological effects of arsenicals that will aid in creating a better risk assessment model for arsenical-induced lung cancer.

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1588 CONCORDANCE IN GENOMIC CHANGES BETWEEN MOUSE LUNGS AND HUMAN AIRWAY EPITHELIAL CELLS EXPOSED TO DIESEL EXHAUST PARTICLES.

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Human and animal toxicity studies have shown that exposure to diesel exhaust particles (DEP) or their constituents affect multiple biological processes including immune and inflammatory pathways, mutagenesis, and in some cases carcinogenesis. This study compared genomic changes by three DEPs with varying extractable organic content in a mouse *in vivo* lung model and a human airway epithelial cell culture system (HAEC). The three DEPs used were from a forklift (NIST SRM 2975 [N-DEP]), a compressor (C-DEP), and an automobile (A-DEP). BALB/c mice were instilled intranasally with saline or 150 μg of DEP on days 0 and 13 and necropsied 18 hrs later. HAECs were exposed to saline or 100 μg/ml of DEP for 4hrs. RNA was isolated from the lungs or cells, labeled, and hybridized to Affymetrix Mouse 430A GeneChips. Gene expression was normalized using Robust Multichip Average Analysis (RMA) and analyzed by Gene Set Enrichment Analysis (GSEA) followed by Ingenuity Pathway Analysis (IPA). Exposure to all the DEP samples caused alterations in genes associated with hypoxia signaling, regulation of peroxisome proliferators, and anti-apoptosis pathways in both mouse lung and HAEC. C-DEP and A-DEP also induced alterations in the Aryl hydrocarbon pathway in both systems. Several other pathways related to acute phase proteins, cell cycling, NFκβ activation, and oxidative stress were significant in all DEP exposed mouse lung tissue. In conclusion DEPs elicited several toxicologic responses in common to both *in vitro* and *in vivo* exposure systems and demonstrated strong concordance between species, while additional effects in mouse lung tissue were complicated by more cell types. This information provides validation for the use of experimental animals in predicting human health effects for respiratory toxicants, while examination of cellular pathways that are altered with varying chemistry will extend biological understanding of DEP-induced toxicity.(Supported by the EPA Training Agreement CT 829472. This abstract does not reflect EPA policy.)

1589 THE URINARY BLADDER EXHIBITS A U-SHAPED GENOMIC DOSE-RESPONSE FOLLOWING SHORT- AND LONG-TERM EXPOSURE OF MICE TO ARSENATE IN DRINKING WATER.

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Several studies have reported increased urinary bladder tumor incidence in populations exposed to inorganic arsenic in drinking water at concentrations of several hundred micrograms per liter, but experimental animal studies at much higher concentrations have been equivocal. Previous studies have indicated that the interactions of arsenic with key proteins and genes in the cell regulatory network change dramatically between low (sub-micromolar) and high concentrations. To investigate the nature of these differences more comprehensively, urinary bladders from mice exposed to inorganic arsenic in drinking water at concentrations ranging from 0.5 to 50 mg/L were analyzed using whole-genome arrays. After 5 days of exposure, the number of genes with significant changes in expression from controls were 15, 0, 302, and 341 at 0.5, 2, 10, and 50 mg/L, respectively. After 12 weeks of exposure, the number of altered genes were 419, 18, 843, and 1822. There was less than 10% overlap in the genes changed at 0.5 mg/L with those changed at 10 or 50 mg/L at either time-point. There was also less than 5% overlap in the genes changed after short- and long-term exposure. At 12 weeks, gene changes at the low concentration were primarily associated with extracellular matrix, cytoskeleton, and morphogenesis, while the high concentration changes were associated with metal binding, regulation of transcription, RNA processing, and dephosphorylation. Benchmark dose-response analysis by gene ontology category was also performed. The lowest benchmark doses for single genes were on the order of 0.7 mg/L, while the lowest benchmark doses for gene categories were 10.7 mg/L at 1 week (for anti-apoptosis) and 4.0 mg/L at 12 weeks (for negative regulation of cell proliferation). In vitro studies with mouse and human urothelial cells are now in progress to support a parallelogram approach for predicting the in vivo genomic dose-response in the human. (This abstract does not necessarily reflect U.S. EPA policy).

1590 CONCENTRATIONS DEPENDENT TRANSITIONS IN RESPONSES OF RAT NASAL EPITHELIUM TO INHALED FORMALDEHYDE.

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Male Fisher 344 rats were exposed to 0, 0.7, 2.0 and 6 ppm formaldehyde (CH_2O) for 5 days/week for up to three weeks. Nasal epithelium was taken from rats after 1, 5, 8 or 15 exposure days. Epithelium was collected from regions similar to sites with tumors in 2-year inhalation studies. Cell proliferation (ULLI; unit length labeling index) was increased after 5 days at 6 ppm. After 15 days, labeling index, but not ULLI, was increased at 6 ppm; there was decreased cell number per epithelial length; and LI showed a tendency to a U-shaped behavior. Gene expression was examined with Affymetrix Rat Genome 230 2.0 arrays. No genes were statistically altered at 0.7 ppm exposures at any times. At 2.0 ppm, 15 genes were significantly altered at 5 days: only half or these were also increased at 6 ppm at 5 days. The pattern of transcript changes, with more significant responses at 2 ppm, was different at day 5 compared with either the 1- or 15-exposure day group, consistent with tissue adaptation in the first week and reduced tissue sensitivity at intermediate CH_2O concentrations by week 3. The genes changed at 2.0 ppm at 5 days tended to have U-shaped dose response curves when examined over a broader concentration range, with dose-related trends for some at 0.7 ppm. Thus, key epithelial changes over time with respect to CH_2O exposures were reflected both in proliferation and in genomic responses. These changes are consistent with qualitatively dif-

ferent tissue responses at intermediate (0.7 to 2.0 ppm) versus high (6 ppm) CH_2O concentrations. The most sensitive responses were in genes associated with cell membrane and structural integrity of the cell. Extrapolations of risks of inhaled CH_2O from responses at high concentrations to lower regions need to account for these transitions in the mode-of-action for CH_2O.

1591 A TOXICOGENOMIC APPROACH FOR IDENTIFYING BIOMARKERS OF DRUG-INDUCED PHOSPHOLIPIDOSIS.

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Phospholipidosis (PLD) is characterized by an excessive accumulation of intracellular phospholipids. In many cases, drug-induced PLD is considered to be an adaptive response and a reversible change; however, if it leads to dysfunction especially in non-regenerative organs such as nervous system, sensory organs and heart, serious adverse events might be induced by these dysfunctions. Therefore, sensitive biomarkers are needed to monitor or predict PLD.

In this study, we tried to identify candidate gene biomarkers by a toxicogenomic approach using peripheral blood and bronchoalveolar lavage fluid (BALF). Amiodarone (1000mg/kg), Chloroquine (300mg/kg), Maprotiline (150mg/kg), Tamoxifen (100, 300mg/kg), Perhexiline (200, 600mg/kg) and Imipramine (200mg/kg), which are known to induce PLD in multiple organs of rat with above doses, were orally administered 6-week-old male Crl:CD rats once daily for 4 days. We conducted histopathological examination of the lung, liver, kidney, spleen and mesenteric lymph node stained with Hematoxylin-Eosin (HE) and counted vacuolated lymphocytes on the peripheral blood smear. In addition, comprehensive analysis of gene expression was performed using peripheral blood and BALF.

Histopathological changes and vacuolated lymphocytes that were considered to be PLD were observed in treated groups. The result of gene expression analysis revealed that expression level of some genes were altered in association with the severity of PLD in the lung and peripheral leukocytes. Among these genes, we identified such genes for which expression level correlated well to the severity of PLD in the lung, on individual animal basis.

These results suggest that clinically useful PLD-specific gene biomarkers can be selected among these candidate genes through a further optimization.

1592 INVESTIGATION OF TOXICOLOGICAL MECHANISM FOR RAT CORNEA OPACITY INDUCED BY ALKYLALKANOLAMINE.

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One of the alkanolamines, 1-dimethylamino-2-propanol (DMAP), induced corneal opacity in human and rats, however, the toxicological mechanism was uncertain. In the present study, to investigate the mechanism of DMAP induced corneal opacity, 1 ml/kg of DMAP were administered to male rats and, after 0.5 and 5 hr of dosing, ophthalmologic examination by slit lump, pathological examination by light and electron microscopes and gene expression analysis using microarray and RT-PCR were performed. At 5 hr after dosing, corneal opacity was observed in all treated rats while there were no remarkable changes in the eye at 0.5 hr. In the microscopic examination, many watery vacuoles of endoplasmic reticulum were observed in the epithelium of cornea, but there were no findings in the other area of eye. In the microarray analysis, 232 genes at 0.5 hr and 526 genes at 5 hr were differentially expressed when compared to the control. In the both time points, ion transporter-related genes (eg. Cl channel) were up-regulated and furthermore, cholesterol biosynthesis-related genes (eg. HMG-CoA reductase) were also up-regulated at 5hr. It is known that Cl channels affect electrolyte balance, fluid transport and cell volume, and the osmic stress for imbalance of Cl concentration induce watery vacuoles. The over expression of HMG-CoA reductase affects morphology and proliferation of endoplasmic reticulum. From these evidences, DAMP induced corneal opacity was due to watery vacuoles of endoplasmic reticulum induced by the imbalance of intercellular electrolytes such as Cl concentration.

1593 TOXICOGENOMICS OF DRUG-INDUCED PROXIMAL TUBULAR INJURY BY ANALYZING GENE EXPRESSION PROFILES IN THE KIDNEY.

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The application of microarray technologies to toxicology, toxicogenomics, is becoming a generally accepted approach for identifying chemicals with potential safety problems. Currently, several efforts to construct toxicogenomics databases have been initiated all over the world. Our Toxicogenomics Project in Japan was a collaborative project by the National Institute of Health Sciences, National Institute of Biomedical Innovation and 15 pharmaceutical companies in Japan. Although the main target of the project is hepatotoxicity, 13 of the 150 chemicals were typical nephrotoxicants or drugs showing clinical side effects [e.g., cisplatin (CSP), carboplatin (CBP), gentamycin (GMC), vancomycin (VMC), phenacetin, bucetin, etc.] and 12 chemicals also exhibited nephrotoxicity in addition to hepatotoxicity (phenylbutazone, ethionine, indomethacin, etc). For these chemicals, gene expression in the kidney was also measured, i.e., 25 chemicals are presently available for analysis of nephrotoxicity. The specific aim of the present work was to develop identifiers for early assessment of potential proximal tubular injury of chemicals based on gene expression profiles in reference to our toxicogenomics database and evaluation systems, named as TG-GATEs (Genomics Assisted Toxicity Evaluation System developed by Toxicogenomics Project, Japan). To define a reliable predictor and novel candidate biomarker genes, we performed gene expression profiling of four prototypic nephrotoxicants, CSP, CBP, GMC and VMC. Expression data were analyzed using prediction analysis of microarrays, and support vector machines algorithms. Its validity was further checked for other chemicals stored in the database. The potential application of toxicogenomics for the identification of candidate genes associated with renal proximal tubular injury caused by chemicals in the rat kidney will be discussed.

1594 EXAMPLE USE OF mRNA PROFILING TO ELUCIDATE MECHANISMS OF CARDIOTOXICITY FOR COMPOUNDS IN PRE-CLINICAL DEVELOPMENT.

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Cardiotoxicity is a common complication in the evaluation of new drug candidates in pre-clinical studies. Numerous examples have demonstrated the applicability of toxicogenomics toward the mechanistic characterization of hepatic toxicities. Far fewer examples have been reported as applied to cardiac toxicities. In our laboratory, we have encountered several cardiotoxic novel molecular entities (NMEs) where microarray analysis assisted with unraveling the underlying mechanism. In one example, Male Sprague-Dawley rats were treated for 1, 3, or 5 days with vehicle or a test article (150 mg/kg/day) undergoing pre-clinical development, which was previously shown to induce cardiac necrosis. Upon sacrifice, the hearts from rats were collected for mRNA profiling and histopathology. No cardiac changes were observed on day 1, but on days 3 and 5, there was evidence of myocardial degeneration and necrosis with secondary inflammatory infiltrates and edema. Using Affymetrix microarrays, the test article induced a significant number of cardiac gene expression changes (1269 probe sets or 8.2% of all probe sets on the array). Placed into biological context, the pathways most impacted involved Ca+2 signals, leukocyte cell adhesion, and a variety of inflammatory pathways. Comparing to a transcriptomic database of over 150 reference compounds including many cardiotoxicants, these profiles correlated closely to those induced by several adrenergic receptor agonists, but not with those induced by other prototypical cardiotoxicants. Thus, impairment of cAMP messaging and G-protein cascades due to possible receptor agonistic activity was proposed as a mechanism responsible for the toxicity. Evaluation of the test article in physiology and pharmacology rat models resulted in hemodynamic effects reminiscent of adrenergic agonism. Thus, toxicogenomics can rapidly generate molecular data useful for hypothesis formulation, which can be interrogated using appropriate follow-up experiments.

1595 DRUG METABOLISM RESPONSE EVALUATED BY ANTIBODY PROTEIN MICROARRAY IN MALE SPRAGUE-DAWLEY RATS EXPOSED TO PHENOBARBITAL (PB), 1, 4 DIOXANE (1, 4-D), TRICHLOROETYLENE (TCE), AND ETHANOL (ETOH).

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Protein microarrays are a powerful and useful tool for high-throughput protein quantification. The aim of this study was to determine the drug metabolism response for several dissimilar chemicals and to compare the PB protein profile to that generated from a microarray study. Rats were dosed (p.o.) daily for five consecutive days with PB (75 mg/kg/day; mkd), 1,4 D (2000 mkd), TCE (1000 mkd), and ETOH (3000 mkd) and evaluated using antibody protein arrays. Additionally, transcript analysis was performed on the same PB exposed rats using Affymetrix Rat 230 2.0 microarrays and the gene response was compared to the protein array response. Following exposure, liver tissue was processed for protein and transcript assays by cytosolic/microsomal and RNA isolation, respectively. The protein array contained 31 probes focused on cytochrome P450, phase II, apoptosis-related, and

oxidative stress proteins. A statistical and 1.5 fold cutoff was used for differential expression for both array types. For the protein array, 4, 7, 18, 19 and 14 differentially expressed proteins were identified for PB, 1,4 D, TCE, and ETOH with each chemical giving unique and characteristic responses with only Calpain, GSTa, and Cyp2e1 being shared amongst all treatment groups. Additionally, 454 differentially expressed genes were identified by microarray analysis from PB exposed animals. Of the 4 proteins identified for the PB group, 3 were also elevated on the Affymetrix microarray: GSTa (1.78 and 2.04 fold), Cyp2b2 (2.71 and 13.23 fold), and Cyp2b1 (3.88 and 14.4 fold, protein and transcript respectively). These data show that protein arrays are useful for generating unique metabolic profiles and aid in validating transcript microarray results.

1596 PROTEOMIC (LC/MS/MS) ANALYSIS OF FFPE RAT LIVER TO IDENTIFY ATORVASTATIN REGULATED PROTEINS: COMPARISON TO FROZEN OCT EMBEDDED LIVER TISSUE.

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Animal/human formalin fixed paraffin embedded (FFPE) tissue archives are invaluable untapped sources of proteomic information. FFPE tissue has not been routinely used for global mass spectrometry (MS) based proteomic studies because formalin fixation causes extensive chemical cross-linking that renders proteins insoluble confounding subsequent extraction, resuspension, separation and analysis. To investigate the feasibility of utilizing FFPE tissue for subsequent MS analysis, protein profiles of FFPE and OCT livers from rats given vehicle or Atorvastatin (300mg/kg for 4 days) were compared. Regulated proteins identified are presented as an average from 5 control and treated liver samples with 2 MS runs per sample. Results demonstrate that 72 and 125 Atorvostatin-regulated proteins were identified in the OCT and FFPE samples, respectively, with 28 overlapping proteins. Regulated proteins consisted of nuclear, cytosolic and membrane proteins indicating broad subcellular representation. Pathway analysis of regulated proteins from FFPE and OCT samples showed similar representation. Signaling pathways affected by Atorvastatin treatment were lipid, amino acid, and carbohydrate metabolism, small molecule biochemistry, drug metabolism and cell-to-cell signaling. Classification of regulated proteins from FFPE and OCT samples into canonical pathways also showed similar patterns that included fatty acid metabolism,glycolysis/gluconeogenesis. Select regulated proteins from FFPE samples were confirmed by western blots. Regulated pathways were consistent with known mechanism of action of Atorvastatin. Results indicate that the number of regulated proteins and identification of affected metabolic pathways are not negatively impacted by formalin fixation of tissue. The number of shared regulated proteins identified from both OCT and FFPE indicate robust proteomic representation of FFPE tissue and global proteomic analysis from archived tissue will be a powerful tool for disease or compound-specific biomarker identification

1597 QUANTITATIVE EXPRESSION PROTEOMICS-BASED SYSTEMS BIOLOGY MODEL OF THE RAT LIVER RESPONSE TO PHENOBARBITAL.

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Phenobarbital (PB) has long been accepted as a prototypical inducer of hepatic xenobiotic metabolizing enzymes. A non-isotopic quantitative expression proteomics-based systems biology model of the rat liver response to PB was developed. Proteins were extracted from the livers of adult male Sprague-Dawley-derived rats following a 5 day i.p. exposure to saline or PB (80 mg/kg/day in saline), differentially detergent fractionated (DDF), and identified using two-dimensional liquid chromatography electrospray ionization tandem mass spectrometry (2D LC ESI MS2) (MudPIT). Of the 3,342 proteins identified, 121 (3.6% of the total) significantly (Bonferoni correction value of < 0.05) increased and 127 (3.8%) significantly decreased when compared to control. The greatest increase was seen for the protein identified as CYP2B2 at about 167 times above control. Eight of the 121 proteins showing significant increases were cytochrome P450s (CYPs). The constitutive androstane receptor (CAR) was also significantly increased at ≈ 5 fold. Other proteins of toxicological interest found to be significantly increased included: microsomal glutathione S-transferase (≈ 49 fold), UDP-glucuronosyltransferase 2B2 and 2B5 precursors (≈ 77 and ≈ 32 fold, respectively), epoxide hydrolase 1 (≈ 56 fold), and hypoxia-inducible factor 1 alpha (HIF-1 alpha) (≈ 26 fold). Proteins showing statistically significant change from control were Gene Ontology (GO) annotated and then modeled using GO and Ingenuity Pathways Analysis (5.0 IPA-Tox™). GO modeling demonstrated a decrease in the production of mitochondria, but an increase in endoplasmic reticulum and the total number of cells. IPA-Tox™ mapped 13 networks. A major node of the top network involved an unspecified monooxygenase linked directly to several CYPs, including the most strongly increased protein, CYP2B2. These analyses demonstrate a high-throughput systems biology approach to assessing toxicant or drug effects in target tissues.(Support:NIH P20-RR17661)

1598 PROFILING OF METALLOTHIONEIN ISOFORMS IN HUMAN PROSTATE CELLS BY MASS SPECTROMETRY.

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Metallothioneins (MTs) are a family of low-molecular weight metal-binding proteins which are consistently accepted by the scientific community as a major weapon in the cell's armamentarium for protection against and recovery from environmental insult. The MTs in humans comprise at least 10 isoforms and each one may perform specific biological functions. The research data derived from a mixture of MT isoforms are hard to be interpreted and might be controversial, thus the methods for characterization of individual MT isoforms are highly desirable. In this work, the authors describe a simple and rapid method using high-resolution matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/TOF) for both identification and characterization of human MT isoforms in cell cultures. The preparation of MTs involves trypsin digestion of cell lysates followed by gel filtration through polyacryamide bead with a mass cutoff of 4000Da (P4). During the digestion, MTs are resistant to trypsin and remain as intact proteins, whereas most co-existing proteins are cleaved into smaller peptide fragments, majority of which are readily removed by p4. Subsequently, the intact accurate masses as well as amino acid sequence information are acquired and used to unambiguously identify the individual MT isoforms as well as their posttranslational modifications. The method has been applied to the study of MTs from RWPE-1 cells under the conditions of exposure to zinc or no exposure. Three MT isoforms have been identified in untreated RWPE-1 cells; these are MT-1E, -1X and -2A, primarily acetylated at the N-termini. Interestingly, the zinc treated RWPE-1 cells express two additional MT isoforms, MT-1F and -1G, again being primarily N-term acetylated. The express of MT-1E, -1X and -2A dramatically increase upon zinc treatment. These results are in agreement with the expression of MT mRNAs in RWPE-1 cells determined by RT-PCR.

1599 PROTEOMIC PROFILING OF CULTURED HUMAN BLADDER CELLS AFTER TRIVALENT ARSENICAL EXPOSURES.

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Chronic exposure to arsenic has been associated with human cancers of the bladder, kidney, lung, liver, and skin. Inorganic arsenic is biotransformed in a stepwise manner via both a reduction and then an oxidative methylation step in which arsenic cycles between +5 and +3 oxidation states. To identify global protein-level changes associated with exposure to the individual trivalent arsenicals, a comparative proteome analysis of human urothelial cells (UROtsa) has been performed. UROtsa cells have been shown not to biotransform arsenic, making them a good model system for study. UROtsa cells treated with 1 microM of arsenite (AsIII), monomethylarsonous acid (MMAIII) and dimethylarsinous acid (DMAIII) for 24h were examined by a 2D difference gel electrophoresis (DIGE) approach and proteins were identified by nanospray-liquid chromatography-tandem mass spectrometry (LC-MSMS) analysis. Exposure to MMAIII showed the most significant changes in the protein expression profiles, exhibiting 64 differentially expressed proteins, including thioredoxin reductase, transgelin, aconitase, cofilin and glyceraldehyde-3-phosphate dehydrogenase. Western blots of a subset of these proteins are being performed to validate the LC-MSMS results. Understanding the effects of these trivalent arsenicals on protein expression may provide information crucial to the understanding of key molecular events underlying their toxicity.
This is abstract does not necessarily reflect EPA policy.

1600 METABOLOMIC PROFILES OF LIVER AND PLASMA FOLLOWING WHOLE-LIVER IRRADIATION IN MICE.

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Treatment of liver tumors with external beam radiation has taken a limited role in oncology due to the poor tolerance of the liver to radiation therapy. Whole-liver radiation doses in excess of 30 Gray lead to potentially lethal effects. To better understand the metabolic changes that precede these events, a metabolomic study was undertaken to determine the metabolomic profiles of liver and plasma following whole-liver irradiation in mice. Samples were collected from untreated, sham-operated, and radiation-treated mice (dosed with 10 or 50 Gray) at 24 hours after treatment. Using an unbiased and non-targeted approach to identify small molecules,

595 chemical entities were detected, including 407 metabolites in liver and 347 metabolites in plasma. Statistically significant metabolomic changes were more numerous and the observed changes were greater in magnitude in samples derived from liver when compared to plasma. By ANOVA, 116 liver metabolites and 32 plasma metabolites represented the most significant and consistent metabolomic changes following whole-liver irradiation. Random forest analyses of liver or plasma data obtained from the four treatment groups correctly classified samples with 90% and 76% accuracy, respectively, compared to 25% accuracy from random guessing. A generalized decrease in free amino acids was observed in liver tissues after irradiation, as were significant changes in nucleotide (purine) metabolism pathways and carbohydrate metabolism pathways. By comparison, plasma samples were more homeostatic and did not demonstrate widespread metabolomic changes. Based on the observed changes in metabolomic profiles of liver and plasma following whole-liver irradiation, several biomarkers have been proposed for the induction of potentially lethal hepatic injury, including ribulose-5-phosphate and glucose-6-phosphate in liver and several metabolites in plasma. Biological data from this study should provide a solid basis for subsequent studies examining metabolic changes in response to radiological insult.

1601 1H NMR-BASED METABONOMIC INVESTIGATIONS OF ACUTE CHOLINE AND METHIONINE DEFICIENCY INDUCED STEATOSIS IN THE RAT AND MOUSE.

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Methionine (Met) and choline (Cho) are precursors to phosphatidylcholine, which is the major constituent of lipoprotein particles that are the primary mechanism for hepatic triglyceride secretion. Elimination of Met and Cho from the mammalian diet results in the onset of non-alcoholic hepatic steatosis. Our objectives were to determine if 1H NMR-based metabolic profiles of urine were altered in a way that reflected the presence and severity of hepatic steatosis induced by the withdrawal of Cho or both Cho and Met from the diet, and to assess the differences in urinary response between the rat and mouse. 6-8 week old Sprague-Dawley rats (n=30) and B6C3F1 mice (n=24) were equally assigned to one of 3 diet groups: control, Cho deficient (CD) or Cho and Met deficient (CMD). Animals were fed the control diet during a one-week acclimatization period, following which, those the CD and CMD groups were placed on their respective diets until the termination of the study at 168 hrs. Urine samples were collected at least daily for 1H NMR–based metabonomic analysis. Exposure of mice to a CD diet for 168 hr produced moderate hepatic vacuolar degeneration in 3/4 animals, while the CMD diet produced a moderate-to-severe lesion in 4/4 animals. By contrast just 1/5 rats exhibited minimal, diffuse centrilobular fatty change with a consistent (5/5) lesion of mild-to-moderate severity only apparent in the CMD group. This variation in response was reflected in pattern recognition analysis of urinary NMR spectra, with the CD and control rats indistinguishable - in contrast to clear differences between control and CD mice, and between the CD and CMD response in both species. In the rat, the major detected response was an increase in urinary formiminoglutamate concentration. In the mouse, the major effects were changes in the excreted concentrations of Cho degradation products such as trimethylamine, dimethylamine and dimethlyglycine.

1602 PRINCIPLE COMPONENTS AND HIERARCHICAL CLUSTER ANALYSES OF HPLC/ECD-DERIVED LIVER METABOLITE PROFILES IN GLUTAMATE CYSTEINE LIGASE TRANSGENIC MICE TREATED WITH ACETAMINOPHEN.

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Acetaminophen (APAP) is a commonly used analgesic that is hepatotoxic to humans and mice when administered in high doses. Low-molecular weight redox active metabolites have been implicated in the pathogenesis of APAP overdose and numerous other toxicants. These metabolites could be important biomarkers of injury. This metabolomic analysis utilized mouse liver samples from studies investigating the effects of gender and glutamate cysteine ligase (GCL) on APAP-induced liver toxicity. GCL, a heterodimeric enzyme comprised of catalytic (GCLC) and modifier (GCLM) subunits, is the rate-limiting enzyme in glutathione (GSH) synthesis.

GSH is protective against APAP overdose. In one study mice differing in GCLM gene copy number, were treated with saline, APAP, or APAP + N-acetyl cysteine. In a second study, mice over-expressing GCLC and GCLM were treated with saline or APAP. Mouse liver samples were collected 6 hours post-treatment and subsequently analyzed for metabolic differences using HPLC-ECD, with either isocratic or gradient elution. Pirouette pattern recognition software was used to conduct Principle Components and Hierarchical Cluster Analyses (PCA/HCA) on the resulting chromatograms. Utilization of different PCA pre-processing techniques produced metabolite profiles with low inherent dimensionalities, and helped to identify principal components (i.e. a few key metabolites) that differentiated groups with respect to treatment, GCL status and gender. These metabolites will be investigated in future studies to determine their relevance to APAP metabolism and liver injury.

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1603 USE OF METABOLOMICS AS A TOOL TO IDENTIFY TOXICOLOGICAL MODES OF ACTION.

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In the events following a toxic insult, genomics analysis is capable of depicting the earliest changes, followed by proteomics and metabolomics. Consequently, metabolomics are closer to "every day" toxicology than the other "omics". A further advantage of metabolomics is that changes are detectable in blood. If the analysis of a great number of individual organs can be replaced by one matrix, then this will provide significant advantages (such as less invasive method, no need to kill animals, possible time course analysis). We have chosen to perform the analysis of blood metabolites in such a way as to minimize the risk of artefacts as well as to achieve a high number of known metabolites. We have also reduced the amount of variation in the biological system as well as during analysis. To investigate the potential of metabolomics to find biomarkers or specific patterns of change, we have analysed the blood metabolome of rats treated with either HPPD inhibitors, a novel class of herbicides, or compounds inducing hemolytic anemia. The results for the HPPD inhibitors demonstrated that a single metabolite, tyrosine, can be used as a biomarker. In addition to tyrosine we also found a specific and selective pattern of change that involved 9 metabolites. Furthermore, we used 4 compounds (mainly aniline derivatives) to determine a pattern for haemolytic anemia. The pattern consisted of only 4 metabolites, which were specific and selective for this toxicological mode of action.

1604 A METABOLOMIC ANALYSIS OF NON-GENOTOXIC HEPATOCARCINOGENS IN THE RAT.

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Metabolomics/metabonomics, the global profiling of metabolites in a tissue, cell or biofluid, has found applications within toxicology both as a screening tool to identify metabolic changes characteristic of toxicity and as a method for gaining insight into poorly understood mechanisms of toxicity. We have used this approach to study the short term effects of exposure to non-genotoxic hepatocarcinogens (NGHCs) in rats.

Plasma, urine and liver extracts were profiled using a combination of ^{1}H nuclear magnetic resonance (NMR) spectroscopy and gas chromatography-mass spectrometry (GC-MS). Multivariate statistics, including principle component analysis and partial least squares discriminant analysis, were used to look for trends and clusters in the data and to identify metabolites responsible for differences.

In this study ten compounds were chosen to cover a range of biological effects in the liver. These were phenobarbital Na salt, chlorendic acid, diethylhexyl phthalate, monuron and methapyrilene HCl which are NGHCs, cinnamyl anthranilate, diethylhexyl adipate, benzophenone and diethylthiourea which are non-mutagenic non-hepatocarcinogens (NMNHCs) and 2-acetyl aminofluorene which is a genotoxic hepatocarcinogen. The biggest effect in the data set was the response of rats exposed to peroxisome proliferator-activated receptor (PPAR) agonists. These rats had distinct metabolic profiles and showed a large increase in the relative concentration of betaine and changes in unsaturated fatty acid synthesis. For all the compounds investigated unique metabolic profiles could be associated with an individual compound allowing good classification. In conjunction with transcriptomics we are currently pursuing the different mechanisms represented by these compounds to identify what is unique to NGHCs as a class of compound.

1605 ION-DIRECTED-SHY APPLIED TO THE METABONOMIC ANALYSIS OF BEA TOXICITY.

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Statistical hetero-spectroscopy (SHY) is an analytical technique allowing integration of NMR and MS data. Here we present Ion-Directed-SHY (ID-SHY) as an extension to SHY, pre-processing the MS data into distinct components, based on retention time and fragmentation. ID-SHY is applied to the metabolic consequences

of dosing of 2-Bromoethanamine (BEA), a model toxin inducing renal papillary necrosis (RPN). RPN is a major cause of renal failure in humans, induced by chronic exposure to non-steroidal-anti-inflammatories.
BEA induces RPN after acute dosing and was administered to rats as a single i.p. dose. Within the study 70 male Sprague-Dawley rats were divided into four groups, 9 animals dosed as with vehicle only; 18 animals dosed at 50 mg/kg; 24 animals dosed at 150 mg/kg; and 18 animals dosed at 250 mg/kg. Urine samples were collected at sequential time points between 2 days pre-dosing and 3 days post. Spectra were acquired by NMR and UPLC-MS, data were imported into Matlab for analysis. The extent of toxicity was assessed by histopathological examination of the renal papilla.
Animals dosed at 50 mg/kg displayed polyuria; at 150 mg/kg animals displayed lesions of the papilla of variable severity and polyuria; at 250 mg/kg lesions of marked severity in all animals and polyuria were observed. ID-SHY linked several BEA metabolites such as 2-oxazolidone; 5-hydroxy-2-oxazolidone; and aziridine in the NMR and MS.
Statistical integration using ID-SHY demonstrates the mutually supportive nature of the two technologies. ID-SHY statistically links signals observed in NMR to those in UPLC-MS while retaining retention time information lost in SHY. By extension, by broadening the range of identifying characteristics of an unknown, SHY can provide a powerful method for combining NMR and MS data, simplifying the identification of unknown metabolites and maximising the data-extraction from any study.

1606 USING GENETIC ALGORITHMS FOR SIMULTANEOUS SELECTION OF SAMPLES AND VARIABLES TO CLASSIFY TOXICITY OF NMR METABOLIC PROFILES.

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Metabolic profiles derived from NMR data are complex. Statistical and machine learning approaches are useful for yielding insights into the metabolic basis for toxicity. Concentrating modelling on key subsets of metabolites and samples can improve the predictive ability of models and techniques such as genetic algorithms (GAs) have shown promise in this area (1). The Consortium for Metabonomic Toxicology (COMET)(2) built a large temporal database of NMR spectra of urine from rats treated with model toxins and stressors. Here we assess the ability of the GA approach to efficiently, simultaneously select sets of samples and variables from the COMET database to build robust, predictive classifiers of toxicity.
We employed 29 studies from the COMET database (split into training and test sets), comprising treatments exhibiting a variety of liver and kidney toxicities. Only timepoints where >50% of the animals showed a response were used. The data was reduced by integrating consecutive regions, resulting in a data set with 205 variables and 869 samples. The GA selected, simultaneously, a subset of samples and variables which could predict liver or kidney toxicity using a nearest neighbour classifier. As GAs are stochastic the evolutionary process was run 50 times for each setting.
The results indicate that using simultaneous sample and variable selection improved performance relative to either method alone (best accuracy 67% on independent test set). Simultaneous selection also halved computation time. Successful classifiers repeatedly selected particular variables, including regions identified as alanine, lysine, glucose and bile acids, indicating that this approach can aid defining biomarkers of toxicity.
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1607 INTERSPECIES DIFFERENCES IN GASTROINTESTINAL REDUCTIVE CAPACITY: IMPLICATIONS FOR EVALUATING RESULTS OF THE 2007 NATIONAL TOXICOLOGY PROGRAM DRINKING WATER STUDY OF HEXAVALENT CHROMIUM.

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To assess the carcinogenicity of ingested hexavalent chromium [Cr(VI)], the National Toxicology Program (NTP) conducted a two-year study in which F344 rats and B63CF1 mice were exposed to concentrations of sodium dichromate dihydrate ranging from 0 to 516 mg/L (0 to 180 mg Cr(VI)/L) in drinking water. The study reported interspecies differences in tumor developments with oral mucosa tumors observed only in rats and small intestinal tumors observed only in mice. Variations in tissue distribution of chromium (Cr) were reported with higher liver concentrations and lower fecal mass of Cr measured in mice than in rats, on a total mass basis and a body weight-adjusted basis. Collectively, these observations implicate a lower tissue dose of Cr(VI) in the small intestines, a higher reductive capacity in the gastrointestinal (GI) compartments, and more effective elimination of Cr in rats than mice. To evaluate the relevance of Cr(VI) carcinogenicity in rodent models, interspecies differences in digestive anatomy and physiology were analyzed relative to humans. Gastrointestinal parameters, particularly anatomical dimensions, morphological features, mucosal gland secretions, transit times, microflora populations, and fermentation processes were compared on an interspecies basis with specific reference to the GI reductive capacity for Cr(VI). Specifically, the higher alkalinity of saliva, lower gastric acidity in the dual-compartment stomach, incomplete gastric emptying, and longer transit times contribute to poorer reductive environments and greater bioavailability of Cr(VI) within the GI compartments of rodents. Such differences in GI reductive capacity should be incorporated into modeling efforts to assess the oral toxicity of Cr(VI) so that anatomical and physiological parameters closely approximating humans can be optimized, and uncertainties associated with interspecies differences can be limited.

1608 THE CORRELATION OF METALLOTHIONEIN ISOFORM EXPRESSION WITH THE DEGREE OF MALIGNANCY IN A SERIES OF RECENTLY DERIVED HUMAN PROSTATE CANCER CELL LINES.

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The expression of metallothioneins has been shown to correlate to various states of malignancy in a variety of human tumors; however it is not clear why or how these genes respond during the tumorigenic process. Recently a family of cell lines, exhibiting a gradation of malignancies, was produced from the normal human prostate epithelial cell line RWPE-1 by N-methyl-N-nitrosourea mutagenesis. This family of cell lines consists of WPE1-NA22 which forms slow growing, non-invasive, well differentiated tumors in athymic nude mice, WPE1-NB14 which forms non-invasive slightly larger tumors with moderate differentiation, and the WPE1-NB26 cell line which forms faster growing, invasive, tumors with a low degree of differentiation. The purpose of the current study was to assess whether the expression of metallothionein isoforms is altered in tumorigenesis and if the expression correlates to the degree of malignancy. All cell lines were grown in culture, harvested for RNA, and levels of metallothionein isoform mRNA were assessed with real-time PCR. The expression of MT-3 mRNA, exhibited a fold increase over that of RWPE-1 from 4.2 ± 0.3 for the lowest malignant line WPE1-NA22, 12.5 ± 3.1 for the WPE1-NB14 line, and 15.7 ± 1.1 for the most malignant line. Other MT isoform mRNAs had an inverse correlation with the degree of malignancy with MT-2A expression decreasing to 0.6 ± 0.1 fold of that of RWPE-1 in the WPE1-NA22 line, decreasing to 0.2 ± 0.04 in the WPE1-NB26 line; MT-1E expression was 0.2 ± 0.02 in the WPE1-NA22 line decreasing to 0.1 ± 0.01 in the WPE-NB26 line. MT-1X expression exhibited a 50.1 ± 3.4 fold tumorigenic induction in the WPE1-NA22 line, with the expression gradually decreasing across the cell lines to a level of 15.0 ± 2.5 fold in the highly malignant WPE1-NB26 cell line. The MT-3 expression profile across these gradually increasing malignant cell lines is similar to that seen in bladder cancer where expression correlates to tumor grade.

1609 NICKEL INDUCES METALLOTHIONEIN IN AIRWAY EPITHELIAL CELLS BY INCREASING INTRACELLULAR ZINC.

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Metallothioneins (MT) are small cysteine-rich proteins involved in zinc homeostasis and protection from heavy metals and oxidative stress. Zinc is the only metal known to directly induce MT expression. In contrast, the signaling mechanisms through which other heavy metals induce MT expression remain unknown. To determine the mechanism by which nickel increases MT2A transcript levels, we exposed human airway epithelial (BEAS-2B) cells to 200μM nickel sulfate and measured MT2A mRNA. Nickel increased MT2A mRNA levels in a time-dependent manner with peak expression occurring 4h after exposure. This increase was, in part, transcriptional, because nickel stimulated transactivation of a luciferase reporter construct regulated by four metal response element (MRE) tandem repeats. To investigate the role of intracellular zinc in the nickel signaling pathway, BEAS-2B cells were pretreated with TPEN, a zinc chelator, prior to exposure to nickel or zinc. TPEN inhibited the nickel-induced MRE transactivation of the luciferase construct and increased MT2A mRNA expression, suggesting that nickel exposure releases intracellular free zinc. Oxidants can induce MT2A through zinc release and we previously found that treating cells with N-acetyl-cysteine (NAC) or ascorbic acid prevented nickel-stimulated intracellular reactive oxygen generation. However, in the current studies, pre-treatment with NAC, but not ascorbic acid, prevented

nickel from inducing MT2A. These data suggest that nickel releases zinc through a thiol dependent, but reactive oxygen independent mechanism to in increase transactivation of MT2A. *Supported by: NHLBI HL065697, HL077763, HL085655; and NIEHS ES015675, ES10638.*

1610 MTF-1 AND CARM1 MAY COOPERATE TO REGULATE METALLOTHIONEIN EXPRESSION AND METAL HOMEOSTASIS.

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Metallothioneins (MTs) are small metal-binding proteins that play an important role in the detoxification of transition metals and maintaining metal homeostasis. MT transcription is inducible after exposure to a variety of environmental stresses and is largely regulated through the interaction between metal-responsive transcription factor 1 (MTF-1) and metal responsive elements (MREs), which are located in the promoters of MT genes. Previous studies have shown that MTF-1 must translocate to the nucleus and bind to MREs in order to achieve transcriptional activation of MT. However, the mechanism by which MTF-1 senses increased intracellular levels of metals and other stresses to initiate this sequence of events is unknown. In an attempt to understand signaling events that can regulate the sensory function of MTF-1, a yeast two-hybrid analysis was performed to identify MTF-1 interacting partners. Twenty one interacting partners were identified that bound to various regions of MTF-1. One of the partners, coactivator-associated arginine methyltransferase (CARM1), interacts with MTF-1 through amino acids 405 – 625, which contain the proline-rich and serine/threonine-rich regions of MTF-1. To determine if CARM1 could protect cells from metal toxicity, wild-type and CARM1 -/- mouse embryonic fibroblasts were exposed to increasing concentrations of zinc sulfate and cadmium chloride. Cell survival was evaluated by a Neutral Red cytotoxicity assay and revealed that CARM1-/- cells are hypersensitive to both zinc and cadmium treatments when compared to wild-type cells. Surprisingly, despite increased cytotoxicity, real time PCR analysis showed that MT-I mRNA levels increased after metal exposure in CARM1 -/- cells to the same level as wild-type cells. Taken together, these initial data may suggest that MTF-1 and CARM1 cooperatively interact to maintain metal homeostasis and protect cells from the toxic effects of zinc and cadmium.

1611 HEAVY METAL RESPONSES OF HUMAN METALLOTHIONEIN ISOFORM GENES.

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Metallothioneins (MTs) are proteins known to be involved in defense mechanisms against heavy metals and reactive oxygen species. In human, more than ten MT isoform genes have been identified, in contrast to much less ones in other mammalian species. These MT genes are not splicing variants but located independently on the human chromosome 16. The increased number of isoforms in human may have some biological significance: for example, isoforms may have been functionally differentiated to deal with various environmental factors in the evolutional process. However, we know little about the functions of the individual MT isoforms. To clarify functional differences between human MT isoforms, we developed a method to determine individual isoform mRNA levels using real-time PCR. By this method, we studied responses of the isoform genes including *MT-1A, -1B, -1E, -1F, -1G, -1H, -2A, -3* and *-4* to metals such as Cd, Zn, Cu and As in HeLa cells. These metals induced all MT isoform genes including *MT-3* and *-4*, except *MT-1A* that showed no response to Cu. Among these isoforms, *MT-2A, -1X* and *-1E* were predominantly induced suggesting these three isoforms play major roles in metal homeostasis in HeLa cells. Although most of the MT isoform genes was induced more efficiently by Zn, Cd than by Cu, As, *MT-1A* was induced preferentially by As. These results suggest a functional differentiation of MT isoforms.

1612 EMERGENCE OF METHYLMERCURY TOXICITY LONG AFTER A PERINATAL EXPOSURE IN METALLOTHIONEIN-NULL AND ITS WILD-TYPE C57BL MICE STRAINS.

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Background: Existence of long "latency" period of toxicity after exposure to MeHg has been known in humans, while animal studies addressing this issue have been scarce. Such existence would substantially affect the risk evaluation of the chemical. Objectives: To demonstrate existence of latency period in a rodent model, in which the toxicity of perinatal MeHg would become apparent only in later life stage. Metallothionein (MT) knock-out mice was included as a potentially susceptible model strain. Methods: Pregnant MT-null and their wild-type C57Bl/6J mice were exposed to MeHg through their diet containing 5 microgram Hg/g during gestation and early lactation period. The behavioral functions of the offspring were examined with frequently used paradigms, including openfield behavior (OPF), passive avoidance (PA), and Morris water maze (MM) at the age of 12-13 and 52-53 wks. Results: At 12 wk, no behavioral effects of MeHg were detected, except for the OPF performance in MeHg-exposed MT-null females. Whereas, at 52 wk of age, the MeHg-exposed groups showed poorer performance both in PA and MM, and their OPF activity differed from the controls. These effects of MeHg appeared exaggerated in MT-null strain than in the wild-type strain. The brain Hg concentration had leveled off by 13 wks. Conclusions: The results demonstrated the existence of a long latency period after perinatal exposure to low level MeHg, in which the behavioral effects emerged long after the leveling-off of brain mercury in the exposed mice. Hence, the initial (or triggering) toxicological event responsible for the late behavioral effects should take place before this leveling-off of the brain mercury.

1613 RELATIONSHIP BETWEEN PLASMA ANTI-METALLOTHIONEIN ANTIBODY AND RENAL DYSFUNCTION IN CADMIUM EXPOSED WORKERS.

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The aim of this study was to investigate the relationship between the level of the plasma metallothionein antibody (MT-Ab) and the renal dysfunction induced by cadmium exposure occupationally. The 89 male workers in a smeltery and 29 healthy doctors in the hospital were selected as the subjects for the exposure and effect assessment. Enzyme linked immunosorbent assay (ELISA) techniques were used to determined the titer of plasma metallothionein antibody. The urine cadmium(UCd), the blood cadmium (BCd) and the occupational cadmium intake (TTCd) served as the exposure indexes while the urinary beta-2-microglobulin (UB2M), N-acetyl-beta-D-glucosaminidase (UNAG) and albumi (UALB) served as the effect markers for the renal dysfunction caused by the cadmium. And both exposure assessment and risk assessment were conducted in cadmium-exposed workers in China. The dose-dependant manner between renal dysfunction and the level of urinary cadmium was found. No significant correlations between the levels of MT-Ab and the external or internal exposure doses of cadmium ($P > 0.05$) could be found, but the levels of MT-Ab showed the positive relationship with UB2M ($r = 0.218$, $P < 0.05$) and UNAG ($r = 0.302$, $P < 0.001$)—in the cadmium-exposed workers. Workers who had high levels of MT-Ab display cadmium-induced tubular nephrotoxicity more frequently than those possessing low levels of MT-Ab; the odds ratio (OR) is 4.2; the 95% confidence intervals is 1.2–14.5 ($P < 0.05$), respectively. This study suggests subjects occupationally exposed to the cadmium that have higher MT-Ab levels more readily develop renal dysfunction. Thus, the levels of plasma MT-Ab could be used as a biomarker of susceptibility to renal dysfunction in occupational cadmium exposure.

Key Words: cadmium; metallothionein; metallothionein-antibody; renal dysfunction; susceptibility.

1614 CAENORHABDITIS ELEGANS GENE, NUMR-1, ASSEMBLES INTO NUCLEAR STRESS GRANULES AFTER CADMIUM TREATMENT.

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Cadmium is a known human carcinogen, yet it is not completely clear how cadmium can affect gene expression. The underlying mechanisms that modulate cellular response to metal exposure are highly conserved. Here we describe a cadmium-inducible gene, numr-1, from Caenorhabditis elegans. Quantitative real time PCR was used to quantify the response of numr-1 to metal treatment. Following exposure to CdCl2, CuSO4, CoCl2, CrO3, NiSO4, AsNaO2, ZnSO4, and HgCl2 numr-1 transcription was significantly up-regulated in response to all treatments. To determine the sub-cellular distribution of NUMR-1, we constructed a NUMR-1::GFP translational reporter. When nematodes were raised in the absence of metals, constitutive GFP expression in adult animals was observed in the nuclei of chemosensory neurons in the head, and a few cells around the vulva and tail. Cadmium stress induced NUMR-1 expression in intestinal nuclei and multiple nuclei within the two bulbs of the pharynx. Furthermore, NUMR-1 localized to punctate foci within the nuclei after cadmium treatment. We propose that these

punctate structures are nuclear stress granules. In cell culture, heat shock factor-1 has been shown to localize to nuclear stress granules following metal treatment. We analyzed the location of NUMR-1::GFP in animals that also expressed an HSF-1::mCherry translational fusion. We found that NUMR-1 co-localized with HSF-1 after cadmium treatment, suggesting that the punctate foci may be nuclear stress granules. Although many components of nuclear stress granules have been identified their function remains poorly understood. Thus, studies in C. elegans will help facilitate an understanding of how and why these structures are formed in response to heavy metal exposure and may provide further insight into how cadmium can induce specific changes in gene expression.

1615 IMMUNOLOCALIZATION OF METALLOTHIONEINS IN LIVER AND KIDNEY OF WISTAR RATS EXPOSED TO CADMIUM.

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The present study investigated the histological localization of metallothionein (MT) in rats exposed to cadmium chloride ($CdCl_2$) via drinking water. 32 Wistar rats, including both genders, were given cadmium chloride in drinking water at the doses of 0, 2.5, 5.0, 10.0mg Cd/kg body weight (BW), respectively, for up to 12 weeks. After sacrifice, paraffin-embedded liver and kidney tissue blocks were sectioned and subjected to quantitative immunohistochemistry for MT localization. Significantly increased levels of immunoreactive MT (*ir*MT) were observed in tissues of rats at the highest dose and there was a dose-effect relationship (r_{liver}=0.910, r_{cortex}=0.878, $r_{medulla}$=0.900). Stronger *ir*MT staining was observed in renal cortex and less in renal medulla and liver. A biphasic rise in the levels of *ir*MT in renal cortex appears due to the induction by zinc and cadmium respectively. These studies demonstrated the histological localization of *ir*MT in various cell types and the relationship to measured tissue levels of metals.

1616 EFFECTS OF LOW LEVEL EXPOSURE TO CADMIUM AND ARSENIC ON THE KIDNEY.

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Cadmium (Cd) and arsenic (As) are widely distributed environmental pollutants. Several studies reported that chronic exposure to cadmium and arsenic caused kidney injuries in human. As one of mechanism, oxidative stress is suggested to play a role in the early process of Cd and/or As induced tubular damage in the kidney. This study was performed to evaluate the significance of urinary biomarkers and role of oxidative stress under the environment of low level exposure to Cd and/or As in general population. Urine samples were collected from 296 adults (89 males and 207 females). We analyzed Cd and As concentrations and kidney biomarkers such as β2-microglobulin, N-acetyl-β-D-glucosaminidase (NAG) activity in urine. Urinary malondialdehyde (MDA) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels were measured as oxidative stress indices. The concentration of Cd was 1.21 (2.91) μg/L, 0.84 (2.48) μg/g creatinine and As was 5.72 (2.19) μg/L, 3.95 (1.73) μg/g creatinine in urine. NAG, MDA, 8-OHdG were positively correlated with Cd and As, both, in urine. The positive relations were also observed between NAG and oxidative indices. These findings suggest that chronic exposure to low level of Cd and/or As might be cause tubular damage in the kidney through oxidative stress in human.

1617 CADMIUM-INDUCED MALIGNANT TRANSFORMATION OF HUMAN BREAST EPITHELIAL CELLS.

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Environmental factors are believed to account for a large proportion of human breast cancer risk. Recent studies suggest that cadmium is potentially estrogenic and may be a possible risk factor for breast cancer. For example, cadmium may act like an estrogen, forming high-affinity complex with estrogen receptor. Thus, this study was designed to determine if the nontumorigenic human breast epithelial cell line MCF-10A could be malignantly transformed *in vitro* by cadmium. MCF-10A cells were continuously exposed to 2.5 μM cadmium and monitored for signs of transformation, such as changes in matrix metalloproteinase-9 (MMP-9) secretion. After 40 weeks of continuous exposure, the Chronic Cadmium-exposed MCF-10A cells (referred to as CC-MCF) exhibited a loss of contact inhibition *in vitro*. The transformed cells also showed a marked increase in MMP-9 secretion (2.5-fold), a phenomenon observed in invasive tumors and linked to aggressive behavior. Malignant transformation was confirmed when CC-MCF cells produced tumors in nude mice. To understand the molecular mechanisms underlying cadmium carcinogenesis, we performed gene expression comparison between CC-MCF cells and passage-matched MCF-10A cells, using real-time PCR and western blot to examine expression of several oncogenes and tumor suppressor genes. Results revealed an increase in *c-myc* and *K-ras* mRNA and protein levels in CC-MCF cells, while *BRCA1* and *BRCA2* expression decreased compared to control cells. Genomic DNA methylation was significantly reduced in CC-MCF cells (by 24%) compared to control. While these results suggest that cadmium may alter genomic DNA methylation, oncogene and tumor suppressor gene expression in the course of transformation, further study is clearly needed to determine the role of these changes in the transformation process, and additional genomic analysis is underway. The ability of cadmium to directly induce malignant transformation in human epithelial breast cells fortifies the concept that the metal may play a role in breast cancer *in vivo*.

1618 THE INVOLVEMENT OF RENAL IRON ACCUMULATION IN THE SUPPRESSION OF ERYTHROPOIETIN PRODUCTION IN RATS WITH CHRONIC CADMIUM INTOXICATION.

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Sponsor: T. Yoshida.

The renal production of erythropoietin (Epo), an erythroid-specific glycoprotein hormone, is suppressed by chronic exposure to cadmium (Cd), one of the toxic heavy metals, leading to anemia. Cd induces functional Epo hypoproduction without renal pathological damge in the early phase, followed by the later destruction of Epo-producing renal tubular cells. Since Epo production is affected by iron and Cd disrupts iron metabolism, we investigated the involvement of iron in Cd-induced functional Epo hypoproduction, using the rats exposed to Cd with or without iron deficiency. We injected rats with saline or Cd at 2.0 mg/kg s.c. twice a week for 1 or 3 months, with normal diet or low-iron diet. The rats of saline with low-iron diet developed month-dependent slight anemia with the increased plasma Epo and the iron concentrations in the liver, spleen and kidney decreased, while the rats of Cd showed severe anemia without elevation of plasma Epo and the increased organ iron. Especially, the rats of Cd with low-iron diet at 3 months had less plasma Epo and more organ iron than those of saline with low-iron diet. The iron kinetic study using ^{59}Fe demonstrated that the iron absorption into the organs was significantly increased in the rats with iron deficiency, and that Cd had a not-significant but slight increasing effect on the iron absorption. These results indicate that Cd would induce iron accumulation in the organs along with Epo hypoproduction. Therefore, we observed the relationships between the plasma Epo levels and iron levels in plasma or organs, all of which showed negative correlation, characterized by "L curve", the excess production of Epo in the lower range of renal iron concentration. This result suggests that there would be a distinct threshold level of iron, especially in the kidney, to produce Epo. In conclusion, Cd-induced iron accumulation in the kidney would be involved in the functional suppression of renal Epo production in chronic Cd intoxication.

1619 STRAIN DIFFERENCES OF CADMIUM TOXICITY IN THE LIVER AND TESTES OF INBRED WISTAR-IMAMICHI AND FISCHER 344 RATS.

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The toxic effect of cadmium (Cd) vary with species, strain and sex in experimental animals, although the liver and testes are prominent target sites. We have previously reported that Wistar-Imamichi (WI) rats are strongly resistant to Cd-induced lethality compared to other strains including Fischer 344 (F344) rats. The present study was designed to further examine the strain differences in Cd toxicity in the liver and testes of inbred WI and F344 rats, and to clarify the mechanistic basis of these differences in sensitivity. A single Cd injection (3.5 mg/kg, sc) caused a significant increase in serum ALT levels, indicative of hepatotoxicity, in F344 rats but did not in WI rats. Testicular hemoglobin levels, indicative of Cd-induced testicular toxicity, in the testes of F344 rats were significantly increased compared to those of WI rats, when the animals were injected with Cd (2.0 mg/kg, sc). Theses results suggest that WI rats refractory to Cd-induced hepatic and testicular toxicity compared to F344 rats. To elucidate the mechanistic basis of this resistance to Cd-in-

duced toxicity, tissue GSH and MT levels in target tissues were determined. No pronounced difference in the GSH or MT levels were observed in either control or Cd-treated rats. However, the accumulation of Cd in the liver and testes after the injection was significantly less in the WI rats than in F344 rats, indicating a biokinetic mechanism for the observed strain differences. When zinc (Zn) was administered in combination with Cd to F344 rats, it decreased Cd contents in the liver, and was protective effect against the hepatic toxicity of Cd. On the basis of these results, we propose the possibility that the Zn transport system may play an important role in the strain difference of Cd toxicity in WI and F344 rats. Further studies will test this hypothesis.

1620 CADMIUM INDUCED ALTERATION OF CADHERIN EXPRESSION IN THE MT-3 TRANSFECTED HUMAN PROXIMAL TUBULE CELL LINE HK-2.

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Cadmium (Cd^{+2}), a heavy metal, is known to induce nephrotoxicity in the kidney involving inflammation and necrosis of the proximal tubule cells. A group of proteins known as metallothioneins can bind heavy metals and can provide protection against their toxic affects. Previous work from this laboratory has shown that the third isoform of metallothionein (MT-3) is expressed in the human kidney in situ, including the cells of the proximal tubules. Overexpression of MT-3 in a proximal tubule cell line HK-2 that lacks its expression restores dome formation in these cells, suggesting that MT-3 may have a role in differentiation and vectorial active transport in the proximal tubule cells.
This is further supported by the demonstration that overexpression of MT-3 in the human proximal tubule cell line HK-2, induces the expression of E -, P -, K -, and KSP - cadherins compared to that of the parent HK-2 cells. The goal of this study was to determine if cadmium had an effect on the expression of E- and N- cadherins. For this purpose, the HK-2 cell line transfected with the MT-3 gene (HK-2 MT-3) was cultured continuously for four passages in cell culture medium containing 0.5 and 1μM Cd^{+2}. The expression of E- and N-cadherin was determined by real time PCR and western analysis. In addition, we also determined the effect of cadmium on dome formation and transepithelial resistance (TER). Our data shows that exposure to Cd^{+2} decreases the expression of E-cadherin in the HK-2 MT-3 cells, whereas it increases the expression of N-cadherin in these cells. Dome formation and TER are also decreased during exposure to Cd^{+2}. In conclusion, the alteration in cadherin expression patterns elicited by Cd^{+2} in the HK-2 MT-3 cells are consistent with that seen in the epithelial-to-mesenchymal transition (EMT) which is often observed in many renal diseases.

1621 LOSS OF GLUTAMATE-CYSTEINE LIGASE MODIFIER SUBUNIT SENSITIZES MICE TO ACUTE CADMIUM TOXICITY.

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High cadmium (Cd) concentrations cause damage to several organs including liver, lung, bone and kidney. Reduced glutathione (GSH) is one of the cell's major defenses against oxidative stress and has been shown to play a protective role against Cd damage in both animals and cultured cells. GSH is synthesized by sequential enzymatic reactions within the cell. In the first and rate limiting step, glutamate and cysteine are ligated by glutamate-cysteine ligase (GCL) to form γ-glutamylcysteine (γ-GC). The GCL holoenzyme is a heterodimer composed of a catalytic (GCLC) and modifier (GCLM) subunit. Whereas GCLC alone can produce γ-GC, GCLM increases its efficiency by decreasing inhibition by GSH and lowering the Km for ATP and glutamate. Cd toxicity, in the context of acute GSH depletion, has been studied in animals and cells treated with chemicals that deplete GSH, such as buthionine sulfoximine. Instead, we used a genetic model of GSH deficiency. *Gclm(-/-)* knockout mice generated in our laboratory show GSH levels that are 15% (liver) and 25-30% (kidney) of that in *Gclm(+/+)* wild-type littermates. To examine the role of GSH depletion in acute Cd toxicity, we compared *Gclm(-/-)* mice and *Gclm(+/+)* littermates. The effects of a single 30 μmol/kg sc injection of $CdCl_2$ vs isotonic saline (0.9% NaCl) given to both genotypes were compared. Animals were given 1 ml of water by gavage 12-h post-injection; blood and 2-h urine collections were taken. Increases in plasma ALT, AST, BUN, and urinary *N*-acetyl-β-*D*-glucosaminidase, suggesting liver and kidney failure, occurred in *Gclm(-/-)* mice with lesser increases in *Gclm(+/+)* littermates. 100% mortality occurred with $CdCl_2$ treatment in *Gclm(-/-)* mice within 18 h vs no mortality in *Gclm(+/+)* littermates even at seven weeks post-injection. The lower GSH levels present in *Gclm(-/-)* mice led to a dramatic sensitization of the animals, suggesting that the protective role of GSH is absolutely essential to prevent acute Cd toxicity.—***Supported in part by NIH grants R01 ES012463 and R01 ES010416.***

1622 ARSENIC SIGNALS THROUGH NADPH OXIDASE TO PROMOTE MOUSE LIVER SINUSOIDAL ENDOTHELIAL CELL DEFENESTRATION AND INCREASED PECAM-1 EXPRESSION.

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Drinking water contaminated with arsenic is a global public health concern, since it is estimated to cause multiple diseases in over 100 million people world-wide. Liver diseases including portal hypertension, non-cirrohic portal fibrosis, and vascular shunting are all associated intake of elevated levels of arsenic. One contributing factor to the development of these diseases is liver sinusoidal endothelial cell (LSEC) capillarization, which is functional loss of fenestrations, development of a basement membrane, and increase surface expression of PECAM-1. Recently, we have demonstrated that mice exposed to elevated levels of arsenic (10-250 ppb) undergo LSEC capillarization through unknown mechanisms. Therefore, we tested the hypothesis that arsenic signals through NADPH oxidase (Nox) to promote LSEC capillarization. Primary LSECs were harvested from mice and exposed to 0.5-5 μM As(III) for 8 h. As in our *in vivo* studies, As(III) exposure caused dose-dependent defenestration and capillarization . These *ex vivo* exposures to As(III) stimulated superoxide generation, as measured by enhanced reduction of dihydroethidium. As(III)-stimulated dihydroethidium reduction was prevented by the superoxide scavenger Tempol or the selective Nox2 inhibitory peptide, gp91-tat. Treatment with Tempol or gp91-tat before As(III) exposure also prevented As(III)-stimulated defenestration and PECAM-1 expression. Finally, pharmacological inhibition of the GTPase activity of the NADPH oxidase subunit Rac1 demonstrated that As(III) stimulated upstream signaling events that were essential for oxidant generation, defenestration, and enhanced junctional PECAM-1 expression. These data are first to demonstrate that low environmental levels of arsenic stimulate LSEC defenestration through a NOX-mediated signaling cascade. *This work was supported by EPA STAR Fellowship FP-91654201 (AS).*

1623 THE ROLE OF FLAVONOIDS IN MODULATION OF THE METABOLISM OF ARSENIC.

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The biotransformation of inorganic arsenic (iAs) in humans produces trivalent and pentavalent methylated arsenicals. The pattern and extent of iAs methylation is critical for the overall toxicity and adverse health effects associated with exposure to iAs. Our previous work showed a strong correlation between the rate of iAs methylation by primary human hepatocytes and the amount of arsenicals retained in cells, suggesting that membrane transporters that regulate the uptake of iAs and/or efflux of its metabolites affect the capacity of human liver, to methylate iAs. Aquaglyceroporin 9 (AQP9) and the glucose transporter GLUT1 have been identified as the membrane transporters that are responsible for the uptake of iAs by mammalian cells. The multidrug resistance-associated proteins (MRPs) and P-glycoprotein (P-gp) have been shown to mediate the efflux of iAs metabolites. Several reports have shown that the expression and activity of all the above transporters can be modulated by a variety of nutrients. We have examined effects of sub-toxic concentrations of two isoflavone compounds, genistein (MRPs and P-gp inhibitor) and phloretin (AQP9 and GLUT inhibitor), on the transport and metabolism of arsenite [iAs(III)] and methylarsonous acid [MAs(III)] in human hepatocellular carcinoma (HepG2) cells. Our data show that GLUT1, MRP2 and P-gp are highly expressed in HepG2 cells. Exposure of HepG2 cells to genistein decreased the accumulation of iAs(III) but increased the accumulation of MAs(III). Phloretin was responsible for an increased accumulation of both iAs(III) and MAs(III). Efflux of iAs and MAs from HepG2 cells was inhibited by both genistein and phloretin, but phloretin was a more potent inhibitor than genistein. In addition, phloretin inhibited the methylation of iAs(III) and MAs(III) by HepG2 cells. These results suggest that flavonoids could play significant role in modulation of iAs metabolism and toxicity in human cells. (This abstract does not reflect EPA policy.)

1624 CYTOTOXICITY OF COMBINED ARSENICALS ON RAT BLADDER EPITHELIAL CELLS IN VITRO.

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Based on epidemiological data, chronic exposure to high levels of inorganic arsenic in the drinking water is carcinogenic to the urinary bladder of humans. It has been shown that the highly reactive, trivalent organic dimethylarsinous acid (DMA^{III})

and monomethylarsonous acid (MMA^{III}) are formed during the metabolism of inorganic arsenic *in vivo*. It is possible that the mixture of arsenicals in the urine of arsenic-treated rats plays a role the urothelial cytotoxicity. The objective of this study was to determine if combining arsenicals increased the cytotoxicity of the individual arsenicals in a rat urothelial cell line. The MYP3 cell line, an immortalized but not transformed rat urinary bladder epithelial cell line, was seeded into appropriate culture wells. Treatment with the combined arsenicals was begun 24 hours after seeding and continued for 3 days. Combinations of arsenicals used were DMA^{III} with arsenite, dimethylarsinic acid (DMA^{V}) or trimethylarsine oxide (TMAO). Combinations of concentrations used were the LC_{50}, one-quarter or one-half the LC_{50} of one arsenical with one-quarter or one-half the LC_{50} of the other arsenical. When cells were treated with one-quarter or one-half the LC_{50} concentration of both arsenicals, the cytotoxicity was approximately the same as when cells were treated with half the LC_{50} concentration or the LC_{50} concentration, respectively, of either arsenical. Treatment with one-quarter the LC_{50} concentration of one arsenical plus the LC_{50} concentration of a second arsenical had similar cytotoxicity as treatment with the LC_{50} concentration of either of the arsenicals. The effect on the cytotoxicity of arsenicals in combination was additive rather than synergistic toward a rat urothelial cell line.

1625 ARSENIC EXPOSURE DECREASES BETA DEFENSIN-1 EXPRESSION.

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Arsenic (As) exposure has been associated with various cancers, including those of the kidney and bladder. Proteomic analysis of biological samples from two human population studies identified the presence of lower levels of human β-defensin-1 (HBD-1) peptides in the urine of individuals exposed to arsenic in drinking water. To further validate these findings, we examined the effect of As exposure upon *HBD1* gene expression *in vitro*. mRNA levels of *HBD1* were quantified in two cell lines following exposure to sodium arsenite (iAs^{III}) and its methylated metabolite, $MAs^{III}O$. Dose-dependent decreases in *HBD1* gene expression were observed in HeLa cells and in the human embryonic kidney derived 293T cell line following treatment with iAs^{III} and $MAs^{III}O$ for 48h. $MAs^{III}O$ was more potent than iAs^{III} in decreasing *HBD1* expression, in agreement with its postulated role as the toxic metabolite of As. These effects on *HBD1* expression occurred at As doses which did not cause an overall suppression of gene expression. HBD-1 is a cationic antimicrobial peptide constitutively expressed in multiple tissues including epithelial cells of the respiratory tract and urogenital system. Renal cell carcinomas have been shown to have decreased HBD-1 expression both at the mRNA and protein levels, suggesting that *HBD-1* may be a tumor suppressor gene for urological cancers. It is therefore plausible that decreased HBD-1 levels play a role in the development of associated urogenital and/or kidney cancers arising from As exposure. *Supported by NIH grant P42ES04705.*

1626 ARSENIC INDUCED AN INCREASE IN ENDOTHELIAL CELL PERMEABILITY: INVOLVEMENT OF CYTOSKELETON SIGNALING.

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Although it has been shown that human exposure to arsenic is related to an increase in inflammation, cardiovascular diseases, and cancers, the molecular mechanisms of its action remains to be elucidated. In this study, we demonstrated that exposure to arsenic caused an increase in cell permeability in human microvascular endothelial cells (HMEC), which is correlated with the remodeling of actin, microtubule cytoskeleton, and the activation of Erk1/2 signaling by the production of reactive oxygen species (ROS). Inhibition of Erk1/2 activities, blockage of actin filament remodeling, or removal of ROS partially suppressed the arsenic-induced permeability increase, indicating the involvement of cytoskeleton, Erk1/2, and ROS signaling pathways in arsenic-induced endothelial cell morphological changes. Furthermore, we found that arsenic treatment disrupted the localization of VE-cadherin at adherens junctions in HMEC and induced its translocation into the nucleus. Moreover, it was found that arsenic-induced VE-cadherin nuclear translocation was inhibited by treatments with ROS scavengers, and actin filament and microtubule inhibitors. Taken together, this study demonstrates that arsenic stimulation induces an increase in cell permeability in HMEC, which involves actin and microtubule cytoskeleton signaling. The results from this study may contribute to dissecting the molecular mechanisms involved in arsenic toxicity.

1627 ROLE OF NA/PHOSPHATE COTRANSPORTERS IN THE CELL MEMBRANE TRANSPORT OF ARSENATE.

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Arsenate (AsV) is the commonest form of the metalloid As in nature. After exposure and absorption, mainly through the intestine, it is distributed to periphery tissues and reduced to arsenite. In spite of the extensive toxicokinetic studies, the molecular mechanisms of AsV translocation through the cell plasma membranes are still unknown, but it is generally accepted that the phosphate transporters are involved. We have analyzed the role of Na-coupled inorganic phosphate (Pi) transporters in the transport of AsV by performing inhibition kinetics in Xenopus laevis oocytes. Rat NaPi2a, b, and c, as well as Pit1 and Pit2 were expressed in X. laevis oocytes by injection of in vitro transcribed cRNA. Competition kinetics of 32Pi uptake revealed that NaPi2a and NaPi2c (renal isoforms) were inhibited by arsenate with a Ki ten times higher (about 1 mM) than the affinity for phosphate, while Pit1 and Pit2 (ubiquitous transporters) were inhibited with a Ki of 3.3 mM. Because the plasma phosphate concentration is 1-2 mM, the Pi transporters are completely saturated (with Km of 50-100 μM), and therefore the actual uptake of AsV through these transporters is negligible. The intestinal NaPi2b transporter was, however, inhibited by AsV with a Ki of only 40 μM. As the concentration of Pi in the intestine is very variable but generally very low, NaPi2b is therefore a good candidate as a major carrier involved in the absorption of AsV through the intestine. In addition, phosphonoformic acid, a classical inhibitor of renal Pi reabsorption, inhibited all transporters with the same intensity as AsV did, but no one was inhibited with dimethyl-arsenate (cacodylic acid). These findings were confirmed using brush-border membrane vesicles of rat small intestine and kidney cortex, and with rat vascular smooth muscle cells using similar radiotracer assays.

1628 TISSUE DISTRIBUTION OF ARSENIC SPECIES IN MICE CHRONICALLY EXPOSED TO METHYLARSONOUS ACID.

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The metabolism of inorganic arsenic (iAs) in humans yields toxic and carcinogenic methyl-As (MAs) and dimethyl-As (DMAs) intermediates. Methylarsonous acid (MAsIII) is the most acutely toxic species of characterized iAs metabolites. Here, we examined the concentrations of As species in urinary bladder, brain, heart, kidney, liver, lung, pancreas, adipose tissue, skeletal muscle, and spleen of male C57BL/6 mice exposed for 8 weeks to MAsIII (0.1, 1.0, 2.5 5.0 ppm As) in drinking water and in control mice drinking tap water. Mice were fed a Purina Rodent Diet (5058) with the total As content of 24 to 66 ng/g. Arsenic speciation analysis was carried out in digested tissue homogenates by hydride generation-atomic absorption spectrometry with cryotrapping for separation of arsines. Exposure to MAs did not affect water consumption or body weights and did not produce any overt signs of toxicity. DMAs was the major metabolite of MAs in all tissues except for the kidney, which retained mostly MAs. Highest concentrations of DMAs were found in urinary bladder. The presence of iAs in tissues of mice varied according to the lot of diet consumed. iAs derived from diet was retained mainly in kidney, heart, lung, and brain. Concentrations of iAs in some tissues decreased with exposure to MAsIII. Regardless of exposure level, the lowest concentrations of all As species were found in adipose tissue. These data suggest that exposures to MAsIII result in accumulation of MAs in the kidney and DMAs in urinary bladder. Thus, interactions of MAs and DMAs with specific molecular targets in these two tissues may represent common mechanisms for adverse effects associated with chronic exposures to both iAs and MAsIII. (This abstract does not reflect U.S. EPA policy.)

1629 TISSUE DISTRIBUTION AND URINARY EXCRETION OF INORGANIC ARSENIC AND ITS METHYLATED METABOLITES IN C57BL/6 MICE FOLLOWING SUBCHRONIC EXPOSURE TO ARSENATE (AS^{V}) IN DRINKING WATER.

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The relationship of exposure and tissue concentration of parent chemical and metabolites over prolonged exposure is a critical issue for chronic toxicities mediated by metabolite(s) rather than parent chemical alone. This is an issue for As^{V} be-

cause its trivalent metabolites have unique toxicities and relatively greater potency compared to their pentavalent counterparts for many endpoints. In this study, dose-dependency in tissue distribution and urinary excretion for inorganic arsenic and its methylated metabolites was assessed in female C57Bl/6 mice exposed to 0, 0.5, 2, 10 or 50 ppm arsenic (as As^V) in their drinking water for 12 weeks . No adverse effects were observed and body weight gain did not differ significantly among groups. Urinary excretion of, arsenite (As^{III}), trivalent monomethyl arsenic (MMA^{III}), dimethylarsinous acid (DMA^{III}), dimethylarsinic acid (DMA^V), and trimethylarsine oxide (TMAO) increased linearly with dose, whereas AsV and pentavalent monomethylarsenic (MMA^V) excretion was non-linear with respect to dose. Total tissue arsenic accumulation was greatest in kidney > lung > bladder>>> skin > blood > liver. MMA was the predominant metabolite in kidney, whereas DMA was the predominant metabolite in lung. Bladder tissue had roughly equivalent levels of inorganic arsenic and DMA, as did skin. These data indicate pharmacokinetic models for arsenic metabolism and disposition need to include mechanisms for organ-specific accumulation of some arsenicals and that urinary metabolite profiles are not necessarily reflective of target tissue dosimetry. (This abstract does not necessarily reflect Agency policy).

1630 THE INFLUENCE OF SELENIUM ON ARSENIC METABOLISM AND GENOMIC METHYLATION OF LEUKOCYTE DNA.

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Inorganic arsenic (InAs) in groundwater is recognized as a public health concern in many countries. The association between arsenic (As) and clinical outcomes may be modified by selenium (Se) concentrations due to a known biochemical antagonism between the two metalloids and to similar biotransformation pathways. Furthermore, animal studies indicate that Se deficiency causes genomic DNA hypomethylation. The effect of Se on As metabolism and genomic DNA methylation has not been investigated in humans.

We conducted a cross-sectional study of 286 adults exposed to As contaminated groundwater in Bangladesh. We assessed plasma Se, As profiles in blood and in urine, and genomic methylation of peripheral blood leukocyte (PBL) DNA. Genomic PBL DNA methylation was determined using the [3H]-methyl incorporation assay.

Using adjusted linear regression models, Se was negatively associated with blood and urinary As concentrations ($p = 0.04$ and $p < 0.0001$, respectively). In blood, Se was negatively associated with %MMA ($p = 0.008$) and the MMA:InAs ratio ($p = 0.03$), whereas it was positively associated with %DMA ($p = 0.02$) and the DMA:MMA ratio ($p = 0.03$). Se was negatively associated with genomic methylation of PBL DNA ($p = 0.003$). Unexpectedly, we found a strong inverse association between Se and plasma folate concentrations ($p < .0001$).

Our results suggest that biological antagonism between As and Se is physiologically relevant to humans. Further, the associations between plasma Se and blood As metabolites imply that Se may play a role in facilitating the secondary methylation step of As via sustaining the activity of thioredoxin reductase, a selenoprotein. The underlying mechanisms and implications of the inverse associations between plasma Se concentrations and both genomic DNA methylation and plasma folate are unclear and warrant further investigation.

1631 SORPTION OF ARSENIC BY FERRIHYDRITE IN A SIMULATED GI MODEL.

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Arsenic is a toxic trace element found in groundwater due to natural and industrial processes. Exposure has been linked to cancers of the bladder, lungs, skin, kidneys, nasal passages, liver, and the prostate. Arsenic in drinking water is a problem in many countries, notably Bangladesh and Taiwan. The purpose of this research was to utilize a simulated GI model to evaluate ferrihydrite's potential to bind As. A variety of clay minerals and synthesized iron oxides including ferrihydrite were screened for their ability to bind As(III), as sodium arsenite, and As(V), as sodium arsenate. After ferrihydrite was demonstrated to be the most effective sorbent for both As species, adsorption isotherms were performed on ferrihydrite. All isotherm data were fit to the Langmuir equation to determine adsorption capacity (Q_{max}). Ferrihydrite bound 95.93% of As(V) and 97.23% of As(III) in the screening studies and had a Q_{max} of 1.288 mol/kg for As(III) and 0.744 mol/kg for As(V). Using a simulated GI model, ferrihydrite was found to effectively adsorb As(V) and As(III) in the stomach and intestine, while levels of dissolved iron remained low (<3.63 ppm). Ferrihydrite (0.25% w/w) protected adult hydra at levels up to 200 times the minimal effective concentration (MEC) for As(III) and up to 2.5 times the MEC for As(V). These experiments confirm that ferrihydrite is a high capacity sorbent of As, and that it is effective at removing As in a simulated GI model. Future work will focus on applying this GI model to evaluate the safety and efficacy of other potential binding agents. Supported by NIEHS P42 ES04917.

1632 INCREASED SKIN CARCINOMA FORMATION IN ADULT MICE ASSOCIATED WITH DISTORTED STEM CELL DYNAMICS AFTER IN UTERO ARSENIC EXPOSURE.

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Inorganic arsenic is a human skin carcinogen of unknown mechanism. In mice and possibly humans arsenic is a transplacental carcinogen. To help define mechanisms, the effects of *in utero* arsenic exposure were studied in Tg.AC v-Ha-ras transgenic (Tg.AC) mice, a strain genetically pre-initiated for skin carcinogenesis. Pregnant Tg.AC mice received drinking water with 0 to 85 ppm arsenic (as sodium arsenite) from gestation day (GD) 8 to18, and at weaning were exposed to the skin tumor promoter 12-*O*-tetradecanoyl phorbol-13-acetate (TPA; 2 μg/0.1 ml acetone, twice/week, topical) until sacrifice at 40 weeks. Arsenic alone did not induce skin tumors, while TPA alone produced cutaneous papillomas and squamous cell carcinomas (SCC). However, compared to TPA alone, prenatal arsenic before postnatal TPA increased SCC multiplicity (SCC/mouse) in male (270%) and female (230%) offspring. Further, *v-Ha-ras* transcript was increased ~3-fold in tumors resulting from arsenic plus TPA compared to TPA alone, and was elevated 2.4-fold in fetal (GD 18) skin after arsenic alone. Since the *v-Ha-ras* transgene appears to reside in skin stem cells, stem cell dynamics were assessed. Expression of *Rac1*, a key gene in skin stem cell self renewal, and the skin stem cell marker, *CD34*, increased 370% and 240%, respectively, in tumors induced by arsenic plus TPA compared to TPA alone, and both were elevated in fetal skin after arsenic alone. Over-expression of Rac1 and increased CD34 positive cells in tumors induced by arsenic plus TPA was confirmed by immunohistochemistry. Expression of *c-myc*, a key factor in stem cell commitment to transient amplifying cells, was unaltered in skin tumors induced by arsenic prior to TPA. Thus, enhanced skin carcinoma formation after fetal arsenic exposure in Tg.AC mice is associated with distorted skin stem cell signaling and population dynamics.

1633 INORGANIC ARSENITE INDUCES DELAYED OXIDATIVE DNA DAMAGE DEPENDENT ON CELLULAR ABILITY TO METHYLATE ARSENIC.

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Inorganic arsenic is a known human carcinogen that induces formation of reactive oxygen species. After arsenite exposure in various systems, increased levels of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dG), a biomarker of oxidative DNA damage, are observed possibly as a prelude to arsenite-induced genotoxicity. However, 8-oxo-dG based methods have been questioned due to the high background signal. Arsenic is methylated by specific methyltransferase, but methylation capacity varies widely with tissue and target cell. Thus, we investigated the role of arsenic methylation capacity on the ability of chronic in vitro inorganic arsenite exposure to induce oxidative DNA damage using the immuno-spin trapping method, which is based on the direct trapping of DNA radicals with 5,5-dimethyl 1-pyrroline N-oxide (DMPO), forming stable DNA-DMPO nitrone adducts, and combines the specificity and sensitivity of spin trapping and immunochemical techniques for the detection of DNA radicals. The methylation-competent rat liver epithelial TRL1215 cell line and the minimally methylating human prostate RWPE-1 cell line were exposed for 1-24 weeks to levels of arsenite in vitro (0.25-5 μM) known to induce malignant transformation in both lines. Results show that long-term arsenite exposure (> 5 weeks) induced a remarkable, but delayed, increase in oxidative DNA damage in TRL1215 cells in a dose-dependent manner. In contrast, at no time did arsenite exposure increase oxidative DNA damage in RWPE-1 cells. Recent works indicate methylated arsenical intermediates could be genotoxic. Our results indicate that oxidative DNA damage induced by low level inorganic arsenic could be potentially linked to the ability to form methylated arsenicals and may be delayed because of a requirement to accumulate a sufficient level of these methylated arsenical intermediates. This is the first work demonstrating a direct link between arsenic methylation, DNA radicals and arsenic-induced genotoxicity.

1634 CYTOTOXICITY, MUTAGENICITY AND CELLULAR LOCALIZATION OF INSOLUBLE DEPLETED URANIUM IN CHO AA8 AND HUMAN LUNG 16HBE14O CELLS.

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The heavy metal uranium is a suspected human carcinogen with both chemical and radiological effects. Superimposed on its possible modes of action is the influence of its solubility. We have previously shown that DU as soluble uranyl acetate (UA) is mutagenic in repair-deficient Chinese hamster ovary (CHO) EM9 cells through direct DNA damage that includes strand breaks and uranium-DNA adducts. The aim of the current study was to compare the toxicity of depleted uranium (DU) in its soluble and insoluble forms. Repair proficient CHO AA8 cells were exposed to DU as soluble UA or as particulate UO2, UO3, or U3O8 for 24 hr. Cytotoxicity was measured by clonogenic survival, mutagenicity was measured at the hypoxanthine (guanine) phosphoribosyltransferase (hprt) locus, and localization of uranium particulates was visualized by transmission electron microscopy (TEM). All three particulate DU species exhibited a dose-related decrease in cell survival with cytotoxicity of UO3 > UO2 > U3O8 >> UA on a micromol/cm3 basis. Similar cytotoxicity results were also observed in the human bronchial epithelial cell line 16HBE14o; however, the human cell line appears to be more sensitive to particulate uranium than the CHO line. Only the U3O8 complex displayed a weak, but statistically significant, mutagenicity at the hprt locus in CHO cells. TEM detected differing distributions of UO2, UO3 and U3O8 particulates within CHO AA8 cells. These high valent particulate depleted uranium oxide complexes model those formed upon burning of depleted uranium metal. Therefore, their enhanced toxicity relative to soluble DU necessitates further investigation to assess the possible risks in humans exposed to insoluble DU. (Supported by NIH Grants CA96320 (DMS), CA096281 (RCL), F31ES014971 (MY), and The Alfred P. Sloan Foundation (MY))

1635 BIOMATERIALS AS DECORPORATION AGENTS FOR RADIONUCLIDES.

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The risk of deliberate release of radionuclides by terrorist activities has prompted a need to improve materials and methods for removing internally deposited radionuclides. Currently, only a few standard treatments are available following radionuclide internalization. In response to this challenge, we are investigating polysaccharide-derived biomaterials as decorporation materials. Chitosan resembles cellulose in molecular form and is composed of linear polymeric molecules of <β>-(1-4) linked amino-D-glucose units. Due to amino functionality, the complexation behavior of chitosan is pH-dependent. Chitosan is a weak base and it has a pKa value for the D-glucosamine base unit of about 6.2 – 7.4. A systematic investigation of *in vitro* binding affinities of a commercially available chitosan oligosaccharide and its building block glucosamine for representative metal ions has been conducted using potentiometric and various spectroscopic techniques. From these studies, the effective binding of cobalt by chitosan oligosaccharide was observed under approximately physiological conditions. Both chitosan oligosaccharide and glucosamine exhibited pronounced affinity for the metal ion neodymium Nd(III), a surrogate for americium Am(III). *In vivo* studies in rats demonstrate that orally administered chitosan hastens the elimination of orally administered Co-60, with a corresponding decrease in deposition of radioactivity in the liver and other tissues. Similarly, oral administration of the biomaterial alginate obtained from seaweed significantly decreased the oral absorption of Sr-85, with a corresponding decrease in Sr-85 deposition in bone. Alginate is a copolymer consisting of covalently linked homopolymeric blocks of (1-4)-linked <β>-D-mannuronate and its C-5 stereoisomer <α>-L-guluronate residues. These studies, combined with low toxicity, ease of administration, and commercial availability, indicate that polysaccharide-derived biomaterials may be uniquely suited for safe and convenient distribution on a mass scale following a terrorist event. (Supported by NIH/NIAID 1R01AI074067-01 and DOE DE-AC05-76RL01830.)

1636 TRACE ELEMENT PROFILES IN SINGLE STRANDS OF HUMAN HAIR.

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Hair has the unique potential to reveal retrospective information about the nutritional status and environmental exposure of subjects. Trace elements are incorporated into hair during the growth process and hair therefore reflects the trace element status of the subject at the time of hair formation. We have analyzed 1 cm long segments of single hair strands (24-36 cm) from five healthy and occupationally unexposed individuals. The analysis of the hair segments requires extreme analytical sensitivity. By using High Resolution Inductively Coupled Plasma Mass Spectrometry (HR-ICP-MS) we achieved profiles of 13 elements in single strands of human hair. We were able to obtain important information about essential as well as toxic elements. One of the subjects in this study was a woman who moved from India to Norway three years ago. The Sr profile of her hair showed a remarkable decrease from a level of 0.9 μg/l three years ago, ending at 0.02 μg/l at the time of sampling. This significant decrease probably reflects a source of Sr exposure in India, possibly the ground water. For other individuals we have been able to show that trace element profiles in hair can be explained by seasonal changes in diet. The information obtained from the trace element profiles of human hair in this study substantiates the potential of hair as a biomarker and shows that information about trace elements even in non-exposed subjects can be obtained.

1637 INVESTIGATING METAL CONCENTRATIONS IN THE TISSUES AND SHELL OF THE BIVALVES *CRASSOSTREA VIRGINICA* AND *GEUKENSIA DEMISSA* IN THE NY HUDSON RIVER ESTUARY AND LONG ISLAND SOUND USING SYNCHROTRON RADIATION.

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Bivalves are shellfish that filter feed by straining suspended food particles in the water. Heavy metals and other contaminants found in the food and silt in the water they filter are absorbed and reflected in their shells and tissues. Many government and non government groups have teamed up to bring bivalves back to the Hudson Estuary and Long Island Sound for their monitoring and filtering capabilities. Mayor Bloomberg's Sustainable Development report for 2007 proposed putting mussel farms in the Hendrix Creeks water treatment plant discharge which would allow the water to be filtered and monitored. Synchrotron radiation because of its detection sensitivity, spatial resolution and multi-element detection can be used to determine the concentrations and locations of contaminating elements. Elements in shells indicate multi-year time span and in soft tissue indicate compounds which will eventually be deposited in the sediment and may be recycled back into the environment when the bivalves die. Shell and tissue samples of the Eastern Oyster, *Crassostrea virginica*, and the ribbed mussel, *Geukensia demissa*, were taken from known contaminated and uncontaminated locations within the NY Hudson Estuary and Long Island Sound. The gill, adductor muscle, foot and digestive track were dissected and analyzed at the Brookhaven National Synchrotron Light Source using the x26a beamline for Ni, Ti, Cr, Zn, Cu, Fe, Mn, Pb, As and Sr at 27 sites. The results show that Ni and Fe tend to be high, Zn was very high at several site and Mn was moderately high. Cu and Pb were surprising low. This work was supported in part by grants 2R25GM06003-05 of the Bridge Program of NIGMS and the CSTEP Program of NYSDOE.

1638 THE CURIOUS HISTORY OF ANTIMONY AND ITS RELEVANCE TO HUMAN RISK ASSESSMENT.

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Antimony is an element present in small amounts in the earth's crust, but is found in water supplies. Ingestion of its compounds can cause severe stomach upset resulting in vomiting in humans, a fact that has been known for centuries. One such compound, tartar emetic, was one of the most widely used drugs of the 18-19th centuries. As a result of extensive human use of antimony in medical treatment, a body of knowledge of the effects and indications for use of antimony compounds can be found today in the pages of 19th to early 20th century textbooks. However, current safe drinking water levels for antimony were developed based on very limited bioassays conducted exclusively on rodents. These bioassays show minor clinical and enzymatic effects at a dose of about 5 mg/kg-day. Rodents are not a good model for the emetic effects of antimony. From the classic textbooks, a dose of 0.2 mg/kg-day was reported to produce emetic effects in children. Even lower doses were used to treat adults and children for a variety of conditions. Although US EPA and WHO have used rodent studies as the basis of their drinking water quality standards, we believe that the human data on antimony effects cannot be discounted, as it is more sensitive and relevant for a human health risk assessment than one based on rodent data. Our review of the historical medical usage of antimony posed a challenge for estimating the minimal effective daily dose because physicians of the era carefully stepped up dosages of tartar emetic until the desired effects were observed. However, tolerable doses derived from this clinical data, with a standard allowance for human variability, compare very well with the U.S. EPA's standard of 6 ppb and the WHO guideline of 20 ppb, with a much smaller uncertainty factor. Our evaluation has shown that human information from a bygone era can be applicable to addressing today's challenges.

1639 REDUCING LEAD LEVELS IN THE CONTAMINATED SOILS BY PHYTOEXTRACTION USING SUNFLOWERS.

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Lead is one of the most common heavy metals found in contaminated soils. To reduce the contaminant level, remedial methods must be employed. Phytoextraction in which plants extract the contaminants from the soil and store those contaminants in their biomass has been used to bioremediate metal-contaminated soils. In this study the use of sunflowers (Helianthus annuus) in the phytoextraction of lead from both an artificially contaminated soil (sandy loam soil) and a superfund soil was examined. The plants were grown in the artificially contaminated soil by supplementation with three concentrations of lead nitrate, and in a superfund soil that has had elevated levels of lead under a controlled experimental condition. The concentrations of lead in the soils before and after the phytoextraction, as well as the lead concentrations in the seeds of the sunflower after the phytoextraction were analyzed using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) according to EPA Method. Results showed that lead was extracted from the sandy loam soil supplemented with 400, 800, 1600 ppm of lead with the extraction efficiencies of 14.70, 21.73, 38.45 %, respectively. However, the extraction efficiency from the superfund soil was very low (1.24%) under the same conditions applied to the sandy loam soil, indicating the complexity of the field soil samples. A negligible amount of lead was found in the seeds of the sunflowers grown in the contaminated soil, which make the seeds possible for production purposes, such as edible seeds and oil, after being used for phytoextraction. Further studies are needed to explore conditions applied to the field soils for the phytoextraction of metal contamination by using sunflowers.

1640 CARDIOVASCULAR AND OTHER HEALTH EFFECTS ASSOCIATED WITH ARSENIC EXPOSURE IN INNER MONGOLIA, CHINA.

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Arsenic exposure is associated with cardiovascular and other health effects. The study objectives were to investigate the mode of action and to assess dose-response relationships of arsenic on cardiovascular, diabetic and carcinogenic effects in Ba Men, Inner Mongolia. Ba Men residents (total 654) exposed chronically from 0.1 to 826 μg/L of arsenic concentrations via drinking water participated in this study. Arsenic exposure was assessed by analyzing arsenic levels in well water, nail and urine. Arsenic effects examined including:(1) ECG QT interval prolongation for cardiovascular effects, (2) hemoglobin A1c for diabetes risk, and (3) DNA fragmentation and chromosome damage, gene expression of DNA repair genes (OGG1 and ERCC1) and human telomerase reverse transcriptase (hTERT), a growth-promoting factor. ECG QTc intervals were determined. QTc interval ≥ 0.45 second was considered to be prolonged. The results showed that prolonged QTc interval was associated with water arsenic levels ($p<0.001$) showing a dose-response relationship. Females were more susceptible to QT interval prolongation than males. Hemglobin A1c was determined in blood samples and showed positive association with water arsenic levels ($p<0.001$). In buccal cells, DNA fragmentation by TUNEL assay was associated arsenic exposure at high levels of arsenic exposure (430-690 μg/L), whereas increased micronucleus frequency (chromosome damage) was associated with arsenic exposure starting at lower concentrations (100-300 μg/L). The mRNA levels for OGG1, ERCC1 and hTERT in blood cells were positively associated with water arsenic levels ($p<0.01$). These results showed that we were able to use the ECG and biomarkers to link arsenic exposure to cardiac effects, diabetic risk and cancer-related effects showing dose-response relationships. (This abstract does not reflect EPA policy.)

1641 NONLINEAR BLOOD LEAD RESPONSE OF CHILDREN TO SOIL LEAD: TOXICOLOGICAL BASIS FOR HEALTH DISPARITIES IN METROPOLITAN NEW ORLEANS, LOUISIANA.

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This research explores the association between blood lead (BL) and soil lead (SL) in Metropolitan New Orleans. The study is unique because it draws on a universal blood lead (BL) screening datasets from years 2000-2005 (n = 55,551) spatially coupled with a soil lead (SL) dataset (n = 5,467) completed in 2000. We evaluated associations between measurements of BL and SL exposure responses of children in New Orleans by stratifying the datasets by Census Tracts and analyzing the results with multi-response permutation procedures. A consistent nonlinear association between SL and BL occurred for 6 years with robust significance (P-values $<10^{-23}$). The mathematical model of the pooled BL datasets for 2000-2005 is: BL = 2.038 + 0.172 × (SL)0.5 (agreement (R) of 0.534, an r^2 of 0.528, and a P-value of 1.0×10^{-211} demonstrating that chance alone does not explain the association. Below 100 mg/kg SL the slope of BL response is a steep 1.4 μg/dL per 100 mg/kg. Above 300 mg/kg the response is a more gradual 0.32 μg/dL per 100 mg/kg. In 1995, 37% of children had an elevated BL (≥ 10 μg/dL) for the most vulnerable poor and predominantly African-American children. During the era of universal screening the prevalence of elevated BL declined to 11.8% for the general population of children. However, the association between BL and SL remains strong and the SL map discloses community variations of potential Pb exposure. If health effects occur at BL ≥ 2 μg/dL, then 93.5% of the children in New Orleans are at risk. These data reinforce the proposal that prevention of childhood Pb exposure must include SL remediation as demonstrated by a recover New Orleans pilot project.

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1642 GENOTOXIC EFFECTS OF INDIVIDUALS EXPOSED TO ARSENIC IN DRINKING WATER, IN MORELOS, MEXICO.

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In Mexico, metal-contaminated water is a very important matter of human health, an example of this problem is the region of Huautla, Morelos where metal extraction industry has been generating for thousand years tons of toxic wastes that are released to the environment which implicates human health hazard. Water pollution in Hautla is a major health problem, because of its high Arsenic (As) levels. As, is known to be a human carcinogen and a genotoxic agent. The comet assay is a well known early biomarker of effect, extensively used for assessing genotoxicity in human biomonitoring studies. Therefore, the current study examines the genotoxic effects of continuous and chronic exposure to arsenic which has been found as a contaminant of fresh water source in the region. Men whole blood samples, who are also known to be contaminated with arsenic, were collected by vein puncture and processed for the single cell gel electrophoresis assay. We found statistically significant differences between all exposed individuals and control group. Also, there was no correlation between arsenic concentration in blood and the level of damage observed in the comet assay. Even though the blood is not one of the target tissues of As, the metalloid was capable of inducing genotoxic damage in lymphocytes of all individuals examined. These results suggest that arsenic-contaminated drinking water induces genotoxicity in human lymphocytes, which is a human health risk. This is the first genotoxic damage report in this particular region

1643 ARSENIC EXPOSURE BY DRINKING WATER IN MORELOS, MEXICO.

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Mining industry contamination is a well known problem worldwide. Morelos, Mexico, is an example of this problem which registers high contamination rates of natural resources. Huautla has been a mining district for many years; these activities ended 10 years ago. The drinking water used by the population comes directly from inside the mine which is storaged into a cistern and distributed to households. So, as a preliminary study, the objectives were to evaluate the presence of metal residues in drinking water, as well as metal concentration in blood samples taken from men from Huautla with more than 10 years of residency who consume water from the mine source and to compare them with men from a different drinking water source. Metals in water and in blood were determined by atomic absorption spectrophotometry. The results indicate the presence of high levels of Arsenic (As) in drinking water (mean ± s.e. 0.152 μg/ml in the cistern and 0.236± 0.003 μg/ml in the mine) these levels indicate that in the cistern, As levels are 15 and 23 times above the maximum permissible level respectively (0.010 μg/ml). As levels in blood were also detected. Also, urine samples were taken in order to identify the type of As metabolites present in these individuals. Taken together these results made us to conclude that people of Huautla are in chronic and constant exposure to high levels of As, a fact that urgently needs further evaluation. Our findings are of public health importance since this is the first report of As human exposure in this particular population and nothing is known about the health impact of this exposure in this region.

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1644 URINARY CREATININE: A PREDICTOR OF ARSENIC METHYLATION AND OF AS-INDUCED PREMALIGNANT SKIN LESIONS.

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Ingested inorganic arsenic (InAs) acquires methyl groups from s-adenosylmethionine (SAM) to generate monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA); impaired methylation is associated with adverse health outcomes. The methylation of guanadinoacetate to form creatine quantitatively consumes more SAM-derived methyl groups than all other methylation reactions combined. Creatine is non-enzymatically degraded to creatinine (Crn) which is freely filtered into urine. Due to the link between Crn biosynthesis and methylation reactions, we wished to determine whether Crn might be associated with As methylation and toxicity.

Crn and As metabolites were assessed in urine in 5 studies in Bangladesh: A) a cross-sectional study of 300 randomly selected participants, B) a randomized trial of folic acid supplementation to 200 folate-deficient adults, C) a cross-sectional study of 164 6-yr children, D) a cross-sectional study of 101 pregnant women and E) a nested case control study of 274 incident As-induced skin lesion cases matched to controls for age, gender and As exposure.

Spearman correlation coefficients between Crn and %InAs for studies A-D were -0.32, -0.49, -0.39, and -0.35, $p < 0.0001$ for all studies; those between Crn and %DMA were 0.30, 0.40, 0.22, and 0.32, $p < 0.0001$ for all except the children's study ($p < 0.01$). Correlations with plasma cystatin C, an independent indicator of renal function, did not mirror these results. Higher Crn was associated with reduced risk for skin lesions (OR 0.6, 95% CI 0.4 – 0.8). Multivariate analyses yielded similar results.

Conclusions. Crn is a strong predictor of As methylation and higher Crn is associated with reduced risk for premalignant skin lesions. Additional studies are warranted to clarify the underlying mechanism and potential therapeutic implications of these findings. Urinary analytes are routinely adjusted for Crn to correct for dilution. With regards to arsenic, and potentially many other urinary analytes, urinary Crn is not an "innocent bystander."

1645 ASSESSMENT OF QTC FORMULAS IN CONSCIOUS DOGS WITH PACING-INDUCED HEART FAILURE (HF).

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Drugs given to people with naturally-occurring diseases have been shown to be more toxic than when given to normals, therefore, it appears reasonable to search for potential toxicity in a model possessing a pathophysiological substrate known to sensitize humans to toxicity. Since a search for potential effects of drugs on ventricular repolarization is so critical, models possessing HF have become increasingly popular to investigate potential toxicity. Prolongation of heart rate (HR) corrected QT interval (QTc) is one of the surrogate markers for potential torsadogenicity of a test article. This study was conducted to determine which correction method best accounts for the effects of changes in the RR interval on the QT interval of conscious, sling-trained dog with HF. Bipolar, transthoracic ECGs were obtained from 18 conscious dogs at baseline and after 12 weeks of rapid ventricular pacing. RR and QT intervals were measured and QTc's were calculated using 3 common algorithms (Bazette, Fridericia, and Van de Water). After 12 wks of pacing dogs with HF confirmed by reduced LV shortening fraction had significantly slower HR's ($p<0.01$) and significantly longer QTc's ($p<0.01$) when compared to baseline. The following regression equations were related QTc to RR: QTc(V)=239.4-2.68RR, r^2=0.00, for the healthy dog; QTc(F)=258.4-0.01RR, r^2=0.01 for the dogs in HF. The Van de Water method demonstrated least dependency of QTc on RR interval in conscious, healthy dogs, but the Fridericia method was best for correcting dogs in HF. In conclusion the rate corrected QT formula of Fridericia appears to be useful as a biomarker for assessing effects of test articles on ventricular repolarization in dogs with HF.

1646 ECG ASSESSMENT OF DRUG-INDUCED PROARRHYTHMIA IN ADULT ZEBRAFISH.

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In this study, we examined drug effects on cardiac proarrhythemia by conventional ECG techniques adapted for performance on adult zebrafish. Proarrhythemia is a drug induced disturbance of cardiac rhythm, and bradycardia, tachycardia, AV block and QT prolongations are some of the significant clinical manifestations. To assess proarrhythemia predictability in the zebrafish model, we tested 7 drugs known to cause QT prolongation in humans: terfenadine, astemizole, thoridazine, haloperidol, quinidine, procainamide and sotalol, and 3 negative QT drugs, verapamil, penicillin and propanolol. ECGs were obtained from anesthetized zebrafish perfused orally with tricaine, a well known fish anesthetic. ECG control baseline was established for 10 min before drug treatment and served as an internal control for each compound followed by 20 min drug treatment. Averaged ECGs were obtained from 30 beats at 1 min intervals to generate a time course of drug effects. From averaged ECGs, RR, PR, QRS and QT intervals were measured and QT was corrected with Fridericia method. Our results showed that zebrafish ECG is highly reproducible. Baseline ECG parameters were similar to previously published results. Terfenadine, astemizole, thoridazine, haloperidol, quinidine, procainamide and sotalol which cause QT prolongation in humans also resulted in QT prolongation in zebrafish. Terfenadine, astemizole, thoridazine, haloperidol, quinidine, sotalol, verapamil, procainamide, and propanolol produced bradycardia in zebrafish. Propanolol, which prolongs PR intervals in humans, resulted in abnormal PR intervals in zebrafish. We have shown that ECG can be reliably obtained from adult zebrafish and that this model system is predictive of human cardiac proarrhythmia.

1647 EVALUATION OF DRUG-INDUCED QT PROLONGATION IN HALOTHANE-ANESTHETIZED MONKEYS AND AGED MONKEYS: EFFECTS OF SOTALOL.

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Cynomolgus monkeys are one of the in vivo models used in safety pharmacological studies to evaluate the effects of drug candidates on the cardiovascular system, especially the ventricular repolarizing phase of the heart. Halothane-anesthetized models, including models using beagle dogs and guinea pigs, have been reported to be sensitive in the detection of drug-induced QT interval prolongation, but little halothane-anesthetized monkey data have been reported. In addition, aging was reported to be one of the factors influencing the QT interval, but aged monkey data have not been available. In the present study, the QT interval prolongation induced by the administration of *dl*-sotalol, a representative class III antiarrhythmic drug, was assessed in 1) halothane-anesthetized monkeys (n=4) using an electrocardiogram and monophasic action potential readings and 2) aged female monkeys (n=7) using a Holter's electrocardiogram. 1) Intravenous administration of *dl*-sotalol at 0.1 mg/kg decreased the heart rate and prolonged the QT interval and monophasic action potential duration. At 0.3 mg/kg, the ventricular effective refractory period and QTc-B were prolonged, and at 1 and 3 mg/kg the PR interval and QTc-F were prolonged, in addition to the effects of the lower dose. 2) The QTc-B of the aged monkeys was longer than that of the younger monkeys, even after the vehicle administration. The difference of the QT interval and QTc between the two groups was expanded after oral administration of 3 mg/kg *dl*-sotalol. In conclusion, 1) the halothane-anesthetized monkey model was considered to be useful to predict drug-induced QT interval prolongation in humans and 2) aging might influence the evaluation of drug-induced QT interval prolongation.

1648 INCIDENCE OF SPONTANEOUS CARDIAC ALTERATIONS IN CONSCIOUS NAIVE CYNOMOLGUS MONKEYS OF MAURITIAN ORIGIN.

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Cardiovascular investigations – among other diagnostic parameters – are essential for the detection and interpretation of untoward findings during pre-clinical toxicity studies. Thus, a characterization of spontaneous variations in the ECG of cynomolgus monkeys is mandatory for the right interpretation of apparent cardiovascular findings. A total of 1632 ECGs recorded from 2000 to 2007 according to baseline intervals (RR, PR,QRS, QT), amplitudes (Q,R,T) and heart axis were analyzed from untreated, unsedated Mauritian cynomolgus monkeys (819M; 813F). Lead II tracings were used and ECG alterations were defined on the basis of in-house reference data for this species. As sex differences proved to be of minor significance (<5%), male and female data were combined. Mostly heart rate (HR) alterations, were due to a slight sinus tachycardia (4.8%) or sinus bradycardia (4.5%). A mild prolongation of the QT- interval (mean QT+ 2SD) and flat or negative T-waves were observed in 2.3% and 1.5% of monkeys, respectively. Severe bradycardia (mean HR as -2SD: 0.9%), conduction impairment such as arrhythmia (0.8%), AVB I (0.8%), AVB II (0.6%) and BBB (0.6%), as well as fused T-P waves (0.7%) were rare as well as ectopic beats (0.1%) or severe tachycardia (0.1%). None of the reviewed ECGs revealed a marked uncorrected or Fridericia-corrected QT- interval prolongation or atrioventricular block of grade III. A normal heart axis was present in 82.1% while other axis deviations (ad) were only apparent in 8.2% (rad), 5.6% (lad) or 4.1% (iad). Surprisingly, during the period 2006 – 2007, sporadic arrhythmias and AVB II, have been increasingly noted with incidences of 3.2% and 2.8%,

respectively. In summary, in this historic ECG database we were able to define baseline values and to observe normal cardiac alterations in Mauritian cynomolgus monkeys. This detailed information will be a further aid for the careful interpretation of cardiovascular electrocardiographic changes during preclinical studies.

1649 COMPARISON OF ANESTHETIC PROTOCOLS FOR UNCONSCIOUS CARDIOVASCULAR SAFETY PHARMACOLOGY IN BEAGLE DOGS.

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Clinical trials have recently revealed that some adverse hemodynamic effets such as hypertension, noted with multireceptor tyrosine kinase inhibitors, may be missed with traditional safety screening tools. This raised an interest for refinement of early preclinical screening tools to assess hemodynamic effects of new test agents even before initiating IND-enabling GLP studies. Volatile anesthetics, e.g. isoflurane, have been reported to induce cardiovascular depression and prolongation of QT interval. In contrast, a premedication of subcutaneous (SC) morphine, followed by alphachloralose IV infusion, has been extensively used for electrophysiological evaluations in anesthetized animal models. Anesthesia using isoflurane (2%) or morphine (2 mg/kg, SC) followed by alpha-chloralose (bolus of 100 mg/kg followed with an infusion at 40 mg/kg/hr, IV) were compared in Beagle dogs under standardized mechanical ventilation. The cardiovascular function of each animal was monitored for at least 3 hours. From a hemodynamic standpoint, a progressive decrease of systemic arterial pressures (systolic, mean and diastolic SAP) was observed with isoflurane anesthesia during extended monitoring periods. All Beagle dogs anesthetized with isoflurane as well as with alpha-chloralose presented a relatively stable heart rate whereas morphine/alphachloralose anesthesia resulted in much lower heart rate (bradycardia). However, dP/dT was more variable with isoflurane (+/-10 to15 sec-1), when compared to alpha-chloralose (+/-4 sec-1), resulting in greater variation of pulse pressure. In conclusion, SC morphine/ IV alpha-chloralose anesthesia in Beagle dogs is considered suitable both for electrophysiological and hemodynamic screening investigations in Beagle dogs and offers more stable baseline data especially when adverse hemodynamic effects occur hours after administration. Isoflurane remains a suitable protocol for hemodynamic and chronotropic effects but the progressive cardio-depressive properties may obscure hemodynamic effects.

1650 POTENTIAL IMPORTANCE OF DETECTING DRUG-INDUCED ALTERATIONS IN REGIOINAL DISTRIBUTION OF CARDIAC OUTPUT.

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The principal goal of the cardiovascular system is to provide perfusion of tissues proportion their needs, therefore measurement of cardiac output, alone, does not provide information on potential deleterious effects of drug-induced changes in regional flow. Injection of neutron-activatable microspheres (uS) that are recoverable from organs or fractions of organs in a number proportional with flow, permits accurate calculation of regional flows during up to 8 times. Healthy dogs were anesthetized with morphine-chloralose and catheters were placed in the left ventricle (for injecting uS) and in the femoral artery for recovering uS (reference sample) from a sink of known flow. With knowledge of the total number of uS injected, the flow rate of the reference sample, and the number of uS removed from the reference sample and from known aliquots of organs, both cardiac output and regional flows were calculated during baseline, and following escalating doses of dobutamine (in 4 dogs) and arginine vasopressin (in 4 dogs). Dobutamine produced a large increase, and vasopressin a large decrease, in cardiac output. Dobutamine produced a large and unexpected decrease in subendocardial myocardial flow, whereas vasopressin produced a large and unexpected decrease in intestinal blood flow. Flow to subendocardium was sufficiently-reduced to result in J-point deviation on the ECG and release of troponin, whereas intestinal ischemia was sufficient to result in mild hemorrhage. Without measurement of regional flows these morbid observation cold not be explained and might have gone undetected. Measurements of cardiac outputs and regional flows by uS technology add approximately $500 in cost per experiment, but contribute valuable information on potential drug toxicity.

1651 THE IMPORTANCE OF EARLY CHARACTERIZATION OF METABOLITES TO ASSESS POTENTIAL CARDIOVASCULAR EFFECTS.

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Compounds which induce QT prolongation in humans commonly act by inhibiting Ikr (delayed rectifier potassium current), which is encoded by hERG (Human Ether-a-go-go Related Gene). Potential development compounds are now routinely screened against hERG channels, along with in vivo cardiovascular assessment, according to ICHS7A/B guidelines. Here, we describe assessment of a novel dual serotonin/norepinephrine reuptake inhibitor to determine potential cardiovascular effects. This compound was a moderately potent hERG inhibitor (IC20 ~ 0.5 μM, IC50 ~ 5 μM), and a sufficient safety margin was predicted. In the dog radiotelemetry study, dose-dependent QTc prolongation (up to 9%) was observed. Surprisingly, the onset of effects occurred at ~ 7-8 hours post-dose, well past the Tmax of the parent (~ 1 hr). A major metabolite was identified (30-40% of the parent in dog), where QTc effects coincided with metabolite exposures in plasma; hERG inhibition (IC20 ~ 0.6 μM, IC50 ~ 6.5 μM) was similar to the parent. In a follow-up study in the isolated guinea pig Langendorff heart model, both the parent and metabolite were tested separately. Interestingly, QT prolongation was observed at lower exposures for the metabolite (3 μM) than the parent (10 μM), as well as increased repolarization dispersion (0.3 vs. 10 μM), suggesting that the metabolite was more likely/potent to induce QT prolongation. Additional studies investigating inhibition of Nav1.5 sodium channels demonstrated that the parent compound was equally potent on hERG and Nav1.5 (IC50 ~ 8 μM), while the metabolite did not significantly inhibit Nav 1.5 (IC50 >100 μM). These results suggest that mixed channel effects may counteract the hERG inhibition for the parent, but formation of a metabolite with primarily hERG effects was more likely to prolong QT intervals. Therefore, the mechanism for the QT prolongation observed in the dog telemetry study at those later timepoints was likely due to formation of this metabolite and highlights the importance of assessing major metabolites as early as possible.

1652 REPEATED TREATMENTS WITH DOXORUBICIN CAUSES ELECTROCARDIOGRAM (ECG) CHANGES AND INCREASED VENTRICULAR PREMATURE BEATS IN WISTAR-KYOTO (WKY) RATS.

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Doxorubicin (DOX) is a widely used anti-neoplastic drug used to treat tumors. However it has been implicated in irreversible cardiac toxicity via the generation of a proxidant free radical, which often results in cardiomyopathy and changes in the ECG. Acute cardiotoxic effects include decreased contractility and electrophysiological abnormalities such as T-wave flattening, and ST and QT elongation. The purpose of this study was to examine the effects of repeated administration of DOX on ECG and several biomolecular parameters in the heart and lungs of WKY rats. Male rats were anesthetized and surgically implanted with radiotelemeters for the continuous monitoring of heart rate (HR) and ECG. Following recovery, rats received either DOX (1.25mg/kg, 2.5mg/kg or 5mg/kg) or vehicle intraperitoneally once a week for three weeks. Rats were sacrificed 24hrs after the final dose for the collection of bronchoalveolar lavage (BAL) and whole blood, and measurement of hearts weights. ECG for each animal was analyzed for the entire duration of the experiment. DOX caused a dose-related decrease in body weight, heart weight, plasma protein, and superoxide dismutase levels, and an increase in BAL protein and albumin. Treatment with 2.5mg/kg and 5mg/kg of doxorubicin caused prolonged QRS, QT and ST intervals, and a decrease in T-wave amplitude. Rats treated with DOX, particularly with 5mg/kg, also had a higher frequency of ventricular premature beats (VPB) and there was evidence of T-wave notching and periods of sinus tachycardia. DOX caused cardiomyopathy resulting in both cardiac and pulmonary changes. These findings suggest that the ECG is a sensitive indicator of oxidative injury to the myocardium and thus altered ECG may have utility as a biomarker of myocardial oxidant effects. Supported by Curriculum in Toxicology/UNC Grant T32-ES07126. (This abstract does not reflect EPA policy.)

1653 CHARACTERIZATION OF CARDIOVASCULAR EFFECTS IN ACID SPHINGOMYELINASE KNOCKOUT MICE FOLLOWING ADMINISTRATION OF RECOMBINANT ACID SPHINGOMYELINASE (RHASM).

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Type A and B Niemann-Pick Disease is a lysosomal storage disease in which a deficiency of the enzyme acid sphingomyelinase (ASM) results in accumulation of sphingomyelin (SPM) in various tissues. During pre-clinical evaluation of recombinant human acid sphingomyelinase (rhASM) at doses above the target therapeutic range, clinical signs of toxicity in ASM knock-out (ASMKO), but not wild type, mice were noted. The effects included severe lethargy, coldness to touch and, in some cases, death (LD50 (10 mg/kg)). In order to further characterize and monitor

these effects, we implemented various physiological techniques including; ECGenie® (Mouse Specifics), which captures electrocardiographs (ECG) from conscious rodents, the CodaTM1(AD Instruments) system which, measures systemic blood pressure, via tail vein occlusion, and finally ex-vivo cardiac function, using the isolated perfused heart preparation (Langendorff). Our results demonstrated that rhASM (>10 mg/kg, IV) elicited biphasic cardiovascular effects in ASMKO mice composed of an initial bradycardia at ~45 minutes followed, by reflex tachycardia and a second phase of bradycardia. Associated with the cardiovascular changes was hypotension apparent at ~120min post-dose. In the isolated ex-vivo heart, perfusion of rhASM in ASMKO hearts appeared to elicit cardiac dysfunction (decreased heart rate and abnormal cardiac relaxation) similar to the in-vivo results. Investigations are underway to determine if the breakdown of SPM, resulting in the release of the bioactive lipid ceramide, is responsible for mediating these effects, since similar observations were not noted in wild-type mice.
In Conclusion, using various physiological techniques, we have further characterized the effects of rhASM, above the target therapeutic range, in ASMKO mice.

1654 SUBCHRONIC INHALATION OF ZINC SULFATE CAUSES CARDIAC CHANGES IN HEALTHY RATS.

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Zinc is a common metal in most ambient particulate matter (PM), and has been proposed to be a causative component in PM-induced adverse cardiovascular health effects. Zinc is also an essential metal and has the potential to induce many physiological and nonphysiological changes. Most toxicological studies employ high levels of zinc. We hypothesized that subchronic inhalation of environmentally relevant levels of zinc would cause cardiac changes in healthy rats. To address this question, healthy male WKY rats (12 wks age) were exposed via nose only inhalation to filtered air or 10, 30 or 100 $\mu g/m^3$ of aerosolized $ZnSO_4$, 5 h/d, 3 d/wk for 16 wks. Necropsies occurred 48 h after the last exposure to ensure effects were due to chronic exposure rather than the last exposure. No significant changes were observed in neutrophil or macrophage count, total lavageable cells, or enzyme activity levels (lactate dehydrogenase, n-acetyl β-D-glucosaminidase, γ-glutamyl transferase) in bronchoalveolar lavage fluid, indicating minimal pulmonary effect. In the heart, cytosolic glutathione peroxidase activity decreased, while mitochondrial ferritin levels increased and succinate dehydrogenase activity decreased, suggesting a mitochondria-specific effect. Although no cardiac pathology was seen, cardiac gene array analysis indicated changes in genes such as ion channels and cell signaling, a pattern similar to that seen following acute pulmonary zinc exposure. These data indicate that inhalation of zinc at environmentally relevant levels may induce cardiac effects. While changes are small in healthy rats, these may be especially relevant in individuals with pre-existent cardiovascular disease. (Abstract does not represent USEPA policy. This research was supported in part by UNC/EPA CT829471.)

1655 MEASUREMENT OF CARDIAC TROPONIN T (cTnT) IN RAT SERUM: COMPARING TWO ELECTROCHEMILUMINESCENT (ECL) PLATFORMS.

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The structural protein, troponin T, is used for the diagnosis of myocardial injury. Normally, cTnT is very low in the circulation. When damage occurs, cTnT is released from myocardial tissue resulting in increased blood concentration. Thus, measurement of serum cTnT is a sensitive and specific marker of cardiac injury. The National Toxicology Program is assessing the use of cTnT as a biomarker of chemically induced cardiac injury in standard toxicology testing protocols. Two ECL platforms for cTnT; BioVeris M1M (BV) and Meso Scale Diagnostics SECTOR™ Imager 2400 (MSD), were evaluated for robustness and precision. Serum was collected from naïve male and female Wistar rats and male rats administered isoproterenol (ISP) (5 mg/kg, sc). In general, inter- and intra-assay precision for both platforms was acceptable (CV ± ~10%). Both platforms were robust with a dynamic range that covered 3-4 logs. Thus, an increase in cTnT of at least 100-fold the normal level could be quantitated without sample dilution. A small matrix effect was observed using both platforms. Using the BV human cTnT immunoassay, the ECL signal in serum collected from naïve rats was below the limit of detection and, therefore, could not be quantitated. In contrast, using the MSD MULTI SPOT® Rat Cardiac Injury Panel, cTnT levels in naïve rats typically fell within the quantifiable dynamic range. The physiological levels of serum cTnT from male and female rats measured using the MSD assay averaged 157 and 196 pg/ml, respectively. cTnT increased in male rats administered ISP to 1.6 and 4.5 ng/ml as quantitated by MSD and BV assays, respectively. The difference between the cTnT levels measured in these assays may be related to the different sources of detection antibody and/or calibrator (rat vs. human TnT) used. The MSD platform appears to be more sensitive than the BV technology, includes rat TnT as a calibrator, and has the additional capability of multiplexing to measure cTnT, cTnI and fatty acid binding protein simultaneously in the same sample. Supported by NIEHS Contract No. N01-ES-35513.

1656 EXAMINATION OF THE CARDIOVASCULAR EFFECTS OF PDE2 INHIBITORS IN THE NEONATAL AND ADULT ISOLATED RABBIT HEART (LANGENDROFF) MODEL.

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The objective of this study was to develop an isolated rabbit heart model to screen potential cardiovascular (CV) effects of phosphodiesterase inhibitors (PDEIs). Previously, the effects of PDEIs were thought to be more pronounced in neonatal hearts (NH), but their effects on adult hearts (AH) have not been fully explored. Initially, we determined that NH and AH responded similarly to erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA), a selective PDE2/adenosine deaminase inhibitor. Both NH and AH demonstrated similar reductions in left ventricular pressure (LVDP), contractility and relaxation, as well as decreases in coronary perfusion pressure (CPP). AH were chosen for further studies because they exhibited a more stable function when compared to NH. The hemodynamic (HD) and electrocardiographic (EC) effects of several PDEIs were examined: Ibudilast, BAY-607550, EHNA and Rolipram. Exposure to all four PDEIs was associated with concentration-dependent decreases in LVDP and CPP. The reduction in CPP possibly due to vasodilation, a known effect of PDEIs, may have accounted for the decline in LVDP in this constant flow model. Decreases in monophasic action potential duration (MAPD) were also seen with all PDEIs. In addition, increases in heart rate with Ibudilast and BAY-607550 were observed. In summary, the adult isolated rabbit heart was sensitive to the direct hemodynamic and electrophysiologic effects of PDEIs. The isolated rabbit heart prefused under constant flow possesses utility as a screen to determine the direct CV consequences of PDEIs in the absence of sympathetic feedback, and electrophysiological effects associated with ion channel modulation of these compounds.

1657 EVALUATION OF SEX DIFFERENCES IN ELECTROPHYSIOLOGICAL PROPERTIES USING CYNOMOLGUS MONKEY CARDIAC MUSCLES.

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It is reported that QTc in women is longer than in men and that women are at greater risk of arrhythmia, such as drug-induced TdP. However, the mechanism of this sex difference is not clear. The purpose of this study was to investigate whether there are sex differences in action potential (AP) repolarization, response to proarrhythmic drugs, and ion channel expression in cynomolgus monkey papillary muscles. [Methods] AP in cynomolgus monkey papillary muscles was measured using the microelectrode recording technique. E-4031, chromanol 293B and nifedipine were used as I_{Kr}, I_{Ks} and I_{Ca} blockers, respectively. Ion channel protein (ERG, KvLQT1 and Cav1.2) expression was analyzed by Western blotting. [Results] Mean APD_{90} in females was approximately 18 msec longer than in males. At stimulation of 1.0 Hz, E-4031 induced prolongation of APD_{90} in females and males in a concentration-dependent manner, and the prolongation ratio of APD_{90} against the pre-application value in males by E-4031 at 1 μM was greater than in females. At stimulation of 0.2 Hz, E-4031 induced marked prolongation of APD_{90}, and E-4031 at 1 μM elicited a significantly higher increase in the incidence of early after depolarization (EAD) and triggered activity (TA) in females than in males. Under low potassium conditions at 0.2 Hz, EAD and TA were induced at a lower concentration of E-4031 in females than in males. Nifedipine induced shortening of APD_{90} in a concentration-dependent manner, and there was no difference in the effect between males and females. The shortening ratio of APD_{90} against the pre-application value by nifedipine at 0.2 Hz was greater than at 1.0 Hz. KvLQT1 and Cav1.2 expression in the left ventricle was higher in females than in males. [Conclusion] In the present study, we provide experimental evidence suggesting sex differences in cynomolgus monkey AP repolarization, which might be attributable to ion channel protein expression differences.

1658 ADULT ZEBRAFISH EXPOSED TO 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) DISPLAY REDUCED MYOCARDIAL REGENERATIVE CAPACITY FOLLOWING VENTRICULAR RESECTION.

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Zebrafish have the unique ability to regenerate injured spinal cord, retina, fins and the heart. Humans and other vertebrate species lack the ability to regenerate damaged cardiac tissue and instead undergo myocardial hypertrophy and display scar formation. This makes the zebrafish an excellent model to understand the molecular mechanisms by which heart regeneration occurs. Environmental contaminants such as TCDD have been found to inhibit the normal healing process and inhibit fin regeneration in adult zebrafish by activating the Aryl Hydrocarbon Receptor (AHR) pathway (Zodrow et al. 2004). To test the hypothesis that TCDD inhibits heart regeneration, adult male zebrafish were exposed by intraperitoneally (i.p.) injection to 70 ng/g TCDD or vehicle (control). 24 h after treatment they were anesthetized and 20% of the ventricle was resected. To measure changes in cell proliferation BrdU was i.p. injected on 11, 12 and 13 days post amputation (dpa) and fish were culled at 14 dpa. This time is sufficient for the epicardium to surround the wound and cell proliferation to reach its maximum to replace lost tissue (Poss et al. 2002). At 14 dpa, TCDD exposed fish showed no signs of the epicardium surrounding the wound and exhibited reduced cell proliferation in the heart compared to the vehicle-treated group. This suggests that the activated AHR pathway interferes with the tissue regeneration process in the heart. The molecular mechanisms of which remain to be elucidated. Supported by NIH grant ES012716 and UW Sea Grant.

1659 A COMMON PATHOLOGICAL AND TRANSCRIPTIONAL RESPONSE OF ZEBRAFISH EMBRYONIC HEART TO VARIOUS STRESSES.

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Various stressors such as chemicals and genetic manipulations are known to cause malformation and circulation failure of the embryonic heart. TCDD and retinoic acid (RA) are such examples of these stressors. Both TCDD and RA treatments lead to a similar "small heart" phenotype in zebrafish embryos, characterized by a small and elongated heart, and severe pericardial edema. Furthermore, microarray analysis identified a common cluster of cell cycle and chromosome assembly related transcripts downregulated in the embryonic heart by both TCDD and RA treatment. Since TCDD and RA act through distinct pathways, this prompted us to postulate that unrelated stressors might lead to embryonic heart failure through the downregulation of a common cluster of genes required for heart growth. To test this hypothesis, we exposed embryos to a set of different stressors: valproic acid, carbaryl, and morpholino knockdown of TBX5. These treatments all produced similar responses, characterized by small and elongated hearts with severe pericardial edema. Microarray analysis showed that treatments by these stressors all lead to downregulation of a cluster of cell cycle and chromosome assembly related transcripts. We postulated that the downregulation of this common cluster might be associated with reduced cardiac myocyte proliferation which would cause a reduction in heart size. Consistent with this hypothesis, anti-phosphohistone3 immunohistochemistry showed that fewer proliferating myocytes in the TCDD treated hearts than that in vehicle control hearts. Taken together, we identified in the zebrafish embryonic hearts a common transcriptional response associated with a common pathological response to various stresses, and we are in the process of elucidating the cause of this transcriptional response.

1660 A CARDIOVASCULAR SAFETY EVALUATION STUDY OF ^{99M}TC SESTAMIBI IN DIFFERENT AGED MALE MONGREL DOGS DURING REST AND TREADMILL EXERCISE.

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^{99m}Tc Sestamibi (TcS) is a myocardial perfusion imaging agent approved for use in adults. This study was designed to evaluate the cardiovascular safety in the pediatric population using different aged dogs either at rest (sling restraint) or during treadmill exercise. Dogs, previously implanted with telemetry devices, at the ages of 3, 6, 10 and >15 months served as surrogates for 5, 10, 15 year-olds and adult humans, respectively.

Dogs were dosed with either the vehicle (0.9% sodium chloride, U.S.P.) or TcS (following decay of a ^{99m}Tc-reacted CARDIOLITE® kit) at 0.028, 0.14 or 0.28 mg/kg (1, 5 and 10x max human dose, respectively) using a balanced Latin-Square crossover design during rest and exercise (except 3 month olds) for each of the age groups.

The vehicle and TcS injections in dogs during rest caused a dosing procedure-related increase in blood pressure that rapidly returned to baseline. In the 3 and >15 month old dogs dosed at 0.28 mg/kg TcS, blood pressures remained elevated by 14 to 19 mmHg, compared to vehicle, between 2 and 4 minutes post-dosing. Treadmill exercise increased heart rate in excess of 150 bpm in all dogs of the three age groups. No other changes in hemodynamic or electrocardiographic parameters were observed. TcS administration did not meaningfully alter the hemodynamic or electrocardiographic parameters from the exercise control. In the 6 month age group, diastolic and mean arterial pressures occasionally differed from the vehicle valves towards the end of exercise but this was not considered meaningful because of the sporadic nature and lack of dose relationship.

Thus, with the exception of a small and transient increase in systemic pressure in the 3 and >15 month old dogs at 0.28 mg/kg during rest, administration of TcS produced no alterations in hemodynamic and electrocardiographic parameters during rest or exercise conditions in any age group of dogs studied.

1661 ANALYSIS OF SEX- AND AGE-RELATED DIFFERENCES IN HEART OF RAT STRAIN FISHER 344 USING OLIGONUCLEOTIDE MICROARRAYS.

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Considerable evidence indicates that there are sex-related differences in cardiovascular disease and heart failure in humans, including age of onset, initial manifestations, and drug response. This variability may be reflective of differential gene and protein expression in the two sexes. To understand these sex-associated differences, gene expression profiles in the heart were examined in both males and females at three different ages in a rat model system. In this study, hearts from both sexes of young (8 weeks), middle age (21 weeks) and old age (78 weeks) Fisher 344 rats were ground into powder under liquid nitrogen and aliquots were used for RNA extraction. Agilent whole rat genome oligo microarrays (4x44k) were used in a one-color experimental design. The feature intensity data for each array was imported into ArrayTrack software that was developed at the NCTR/FDA. The raw data were normalized using a 75 percentile scaling normalization method. The Welch T-test was used for determining significant changes in gene expression between the two sexes and between the ages. Principal component analysis showed that there was great similarity in terms of gene expression patterns between the two sexes at different ages. The sex difference was much greater at 21 weeks compared to 8 weeks and 78 weeks. Few common differentially expressed genes at different ages indicated that the sex difference is age-specific. There were 820 differentially expressed genes between the male and female at 21 weeks. Many genes are involved in cell growth, cell cycle, tissue development, and cardiovascular system development. Expression of some energy metabolism genes (ATPase and mitochondrial genes) was also significantly higher in female rats than in male rats. These results may provide insights into sex- or age-related cardiovascular diseases and drug responses.

1662 INVESTIGATION OF CARDIAC SUBENDOCARDIAL PROLIFERATION IN THE RAT AND THE RELEVANCY FOR HUMAN RISK ASSESSMENT.

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A growing number of drugs, including fenfluramine and the ergolines pergolide and cabergoline, cause valvular heart disease in humans. Although not conclusive, evidence suggests that these compounds exert their pathological effect through agonism of 5-HT2 receptors, such as 5-HT2A and 5-HT2B, inducing fibroproliferative plaques overlying normal valve structures and interfering with function. Therefore, drug candidates are frequently screened for activity against 5-HT2 receptor subtypes in order to reduce the likelihood of valvular heart disease. Here we describe a candidate compound that after 5 days of repeat dose administration induced subendocardial proliferation in the left ventricle, affecting the papillary muscle, chordae and mitral valve. The histological appearance of this rapidly developing lesion in rats led us to speculate that activation of the 5HT-2 signaling complex was eliciting the pathologic effect. However, in vitro evaluation of the parent molecule and it's 4 major metabolites screened against 5-HT2 receptors indicates they were all inactive as agonists or antagonists against the 5-HT2A receptor. A 5-HT2B receptor binding and functional assay, using human 5-HT2B receptor stably transfected in CHO and HEK cells, indicated the parent molecule had a Ki of 5 uM and an EC50 of 30 uM. However, functional assays revealed the metabolites were virtually devoid of agonist activity, thus indicating that neither 5-HT2A or 5-HT2B receptors are involved in the subendocardial proliferation in the rat. Amino acid sequence alignment indicates the rat 5-HT2B receptor shares 81% identity to the

human receptor, which indicates the potential for differences in binding characteristics that must be considered. Given the lack of concordance between valvulopathy findings in the rat and in humans following exposure to a variety of compounds, functional differences in 5-HT2B receptor signaling, compound binding properties and metabolism need to be considered to judge the relevancy of preclinical findings.

1663 ANGIOTENSIN II-INDUCED CARDIAC APOPTOSIS IS MEDIATED BY P53-DEPENDENT MITOCHONDRIAL CYTOCHROME C RELEASE PATHWAY.

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Our previous studies showed that angiotensin II (Ang II)-induced cardiac apoptotic cell death and nitrosative damage plays a critical role in the development of diabetic cardiomyopathy. Although tumor suppressor gene p53 was implicated in the pathogenesis of Ang II-induced cardiac cell death, the direct causative role of p53 in Ang II-induced cardiac apoptosis and its signaling pathways have not been defined. In the present study, therefore, cardiac H9C2 cells were exposed to Ang II at 100 nM for 24 hr in vitro, which increased the total and phosphorylated p53 expressions. Interestingly, Ang II induced a time-dependent translocation of p53 from cytosol to mitochondria and then to nucleus. We found that exposure of H9C2 cells to Ang II also induced significant increases in protein nitration, measured by 3-nitrotyrosine (3-NT), and apoptotic cell death, determined by DNA fragmentation. Furthermore, mitochondrial permeability, detected by fluorescent staining and flow cytometry, was significantly increased along with an increased mitochondrial cytochrome c release, determined by Western blotting assay. All these effects of Ang II were inhibited by p53 inhibitor pifithrin-a (PFT-a). To validate the in vitro finding into in vivo condition, FVB mice were given subcutaneously injection of Ang II at the dose of 1 mg/kg body weight with and without intraperitoneal injections of PFT-a at 2.2 mg/kg body weight half of an hour before and 1 hour after Ang II treatment. Expressions of both total and phosphorylated p53 were increased in Ang II-treated WT mice, but not in Ang II/PFT-a-treated WT mice. Ang II-induced capase-3 activation and 3-NT accumulation in the hearts were also prevented in and Ang II/PFT-a-treated WT mice. These results suggest that Ang II-induced cardiac apoptosis is mediated predominantly by p53 activation-dependent, mitochondrial cytochrome c release pathway (Supported by ADA and AHA grants).

1664 CHOLESTEROL SECOALDEHYDE-INDUCED APOPTOSIS IN CARDIOMYOCYTES: ROLE OF HYDROGEN PEROXIDE AND P38 MAP KINASE SIGNALING.

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Cytotoxicity of oxysterols has been extensively studied. Cholesterol secoaldehyde (ChSeco), an "ozone-specific" oxidation product of cholesterol, has generated enormous interest in recent times due to its probable involvement in the pathogenesis of Alzheimer's and cardiovascular diseases. Studies from our laboratory have demonstrated that ChSeco induces apoptosis in rat H9C2 cardiomyocytes, mediated primarily by reactive oxygen species (ROS). The present study was undertaken to establish the type of ROS involved in ChSeco-induced apoptosis. When catalase (10-100 units/mL) and deferoxamine (10-100 μM) were simultaneously added, the cytotoxicity of ChSeco (10 μM; $IC_{50} = 8.9 \pm 1.29$ μM, n = 6) could be completely prevented. In assays using deferoxamine, the intracellular generation of H_2O_2 or H_2O_2-like compounds, measured based on DCF fluorescence, was also found to be inhibited. Pretreatment with N-methylarginine (500 μM; 1 h) did not alter the cytotoxicity, meaning that intracellular generation of nitric oxide may not play a significant role in the observed cytotoxicity. Treatment with SB203580 (p38 Map Kinase inhibitor) and MEK1/2 (inhibitor of ERK1, JNK, and PKC) offered protection against ChSeco-induced cytotoxicity. Taken together, these observations suggest that H_2O_2 generated in response to ChSeco treatments causes apoptotic signaling through the p38 MAP kinase pathway. Studies are underway to investigate the possible role of free iron and the hydroxyl radical formation in cardiomyocytes exposed to ChSeco. [Funding support from NSF (grant number HRD 0450375) and NIH (grant number P20 RR16456) to RMU is acknowledged; Corresponding author, e-mail: rao_uppu@subr.edu.]

1665 AKT-DEPENDENT DOWN-REGULATED GLYCOGEN SYNTHESIS KINASE-3β PLAYS A CRITICAL ROLE IN DIABETES-INDUCED INTRAMYOCARDIAL LIPID ACCUMULATION AND INFLAMMATION: PREVENTION OF METALLOTHIONEIN.

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Yuehui Wang, Lu Cai, Medicine, University of Louisville, Louisville, KY.

Cardiac-specific metallothionein(MT)-overexpressing transgenic(MT-TG)mice were highly resistant to the development of diabetic cardiomyopathy.Evidence indicates that lipotoxicity is associated with inflammatory effects and oxidative damage,leading to the development of diabetic cardiomyopathy. The present study was aimed at investigating the signaling mechanism by which MT protects the heart from lipid accumulation and inflammation.Both MT-TG and wild-type(WT)mice were given a single dose of streptozotocin to induce hyperglycemia,then lipid accumulation and inflammation were examined at different times after the onset of diabetes.To explore the mechanisms,Akt and GSK-3β and glycogen synthase(GS)were also examined.Results showed that at different times of diabetes,lipid accumulation and inflammatory cytokines (TNF-α, PAI-1 and ICAM-1) were significantly increased in the heart of the WT diabetic mice, but not in the MT-TG diabetic mice.Abundance of total Akt,GSK-3 or GS was not significantly changed,but Akt phosphorylation was decreased in the heart of WT diabetic mice compared to the control mice.Decreased phosphorylation of Akt in the hearts of WT diabetic mice was accompanied by a decrease in phosphorylation of GSK-3β,leading to the increased phosphorylation of GS and inhibition of glycogen synthesis.However,neither phosphorylation of Akt nor GSK-3β kinase was decreased in the MT-TG diabetic mice. Administration of GSK-3β inhibitor to WT diabetic mice for two months significantly prevented diabetes-induced cardiac lipid accumulation,inflammation and fibrosis.Therefore,these results suggest that Akt-dependent down-regulated GSK-3β plays a critical role in diabetes-induced intramyocardial lipid accumulation and inflammation. MT prevents diabetes-induced increases in cardiac lipid accumulation and inflammation, through preservation of the Akt-dependent glucose metabolism (Supported,in part,by research grants from ADA,JDRF and Philip Morris USA,Inc.).

1666 FUNCTIONAL STUDY OF CYSTATIN C IN CARDIAC EXTRACELLULAR MATRIX REMODELING.

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Cardiac fibroblasts (CFs) play a central role in the maintenance of extracellular matrix (ECM) in the normal heart and as mediators of inflammatory and fibrotic myocardial remodeling in the injured and failing heart. Numerous lines of evidence have shown that the balance of Matrix metalloproteinases (MMPs) and their inhibitors, TIMPs, plays a critical role in myocardial ECM remodeling. However, the involvement of Cysteine proteinases, for instance Cathepsin B (CTB), and their endogenous inhibitors, such as Cystatin C, in myocardial ECM remodeling has not been well studied.

Our previous finding that oxidative stress induces Cystatin C led us to investigate a novel pathway regulating cardiac ECM with CFs in culture, increased levels of ECM protein and decreased levels of CTB protein and activity were detected upon Cystatin C treatment. To see if Cystatin C can cause ECM accumulation through CTB inhibition *in vivo*, coronary artery ligation induced Myocardial infarction (MI) mouse model was applied. Non-infarcted and infarcted areas were isolated from the MI heart to perform Western blot analysis and CTB activity assay. Increased levels of Cystatin C and ECM protein and decreased levels of CTB protein and activity were detected in the infarcted area comparing to the non-infarcted area of the myocardium. This study, for the first, established Cystatin C as a potential fibrotic factor during myocardial remodeling.

1667 EFFECTS OF AGING ON THE CARDIOVASCULAR AND THERMOREGULATORY RESPONSE TO TOLUENE IN THE BROWN NORWAY RAT.

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Since the proportion of aged in the U.S. will expand markedly for the next several decades, the U.S.EPA is assessing if the aged are more susceptible to environmental toxicants. The neurotoxicity of toluene (TOL) has been well characterized in young adults but has not been studied in aged rodents. To this end, heart rate (HR), HR variability (HRV), core temperature (Tc), and motor activity (MA) were monitored by radiotelemetry (Data Sciences, Int) in young adult (4 m), middle age (12 m) and senescent (24 m) Brown Norway (BN) rats. After 7 d of baseline monitoring in their home cages, the rats were administered TOL (0, 0.3, 0.65 and 1.0 g/kg; oral) using a cross-over design with 7 d between treatments. TOL elicited significant elevations in HR and MA and reductions in Tc. The TOL dose needed to alter HR and Tc was affected by age. There was a rightward shift in the HR dose-response in the senescent rats. TOL-induced hypothermia was greater and more prolonged with age. Baseline HR and MA were unaffected by age but daytime Tc was elevated in senescent animals. TOL increased MA but with no age interaction. Baseline HRV was elevated in senescent rats but there was no effect of TOL treatment or TOL interaction with age. The attenuated tachycardia and prolonged hypothermic response in senescent rats suggests that aging impedes recovery of autonomic processes to a toxic insult. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

1668 GROWTH ARREST, DNA REPAIR, AND TRANSCRIPTOMIC RESPONSE TO DNA DAMAGE IN *CAENORHABDITIS ELEGANS.*

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Caenorhabditis elegans is an important in vivo model for the study of aging, neurodegeneration and carcinogenesis. However, DNA repair itself is less well-characterized in *C elegans*. We adapted a quantitative PCR assay for DNA damage and repair to *C elegans*, and found that the important DNA repair pathway nucleotide excision repair (NER) in this species is comparable genetically and kinetically to NER in humans (Meyer *et al.*, Genome Biology 2007).
To begin to test the importance of DNA repair to adult *C elegans*, we measured lifespan, growth, and feeding in wild-type and *xpa-1*(ok698) NER repair-deficient adult nematodes under control conditions and after chronic exposure to ultraviolet C radiation (UVC). Young adults were exposed to daily doses of 0, 6, 12, 25, 50, or 100 J/m^2 UVC. In the absence of stress, lifespan, growth, and feeding in *xpa-1* nematodes were not detectably different than in wild-type nematodes. Chronic UVC exposure decreased lifespan in both strains in a dose-dependent fashion, but *xpa-1* nematodes were much more affected. Adult growth was inhibited by UVC in wild-type nematodes at high doses, but completely blocked by the lowest dose in *xpa-1* nematodes. UVC exposure also greatly inhibited feeding in *xpa-1* adults, but only mildly affected feeding in wild-type adults. Thus, NER is required for normal adult growth, feeding and lifespan in *C elegans* under conditions of genotoxic stress.
We are currently characterizing the gene expression response to UVC in C elegans of three strains: wild-type, *xpa-1*, and *glp-1*, which is devoid of germline cells at 25° C. These comparisons have allowed us to compare the global gene expression response to UVC in normal, repair-deficient, and germline deficient animals; our results indicate that germline and somatic cells show very different responses to genotoxic insult. We will present detailed analysis of these differences, based on the bioinformatic programs GeneSpring, GOMiner, and Cytoscape.

1669 VALIDITY OF *GPT* DELTA TRANSGENIC RODENT GENOTOXICITY ASSAYS.

T. Nohmi. *Division of Genetics and Mutagenesis, National Institute of Health Sciences, Tokyo, Japan.* Sponsor: A. Nishikawa.

Genotoxicity of chemicals such as pharmaceuticals are usually evaluated by two in vitro and one in vivo assays. The in vitro assays include bacterial mutation assay (Ames) and either chromosome aberration assay with cultured mammalian cells (CA) or mouse lymphoma assay (MLA). The in vivo assay includes mouse micronucleus assay (MN). Since MN identifies chromosome aberrations but not point mutations, no appropriate in vivo assay that can evaluate the significance of Ames assay has been formulated. In addition, recent publication suggests high rates of false positives in CA and MLA (Kirkland et al., Mutat. Res., 584, 1-256, 2005). Thus, it is desired to include an additional in vivo assay in a battery of genotoxicity tests to evaluate the results of in vitro assays. We have developed novel in vivo genotoxicity assays with transgenic mouse and rat (*gpt* delta TG assays). The assays enable us to identify deletion mutations as well as point mutations in vivo, thereby providing useful information about the validity of in vitro assays. Unlike other transgenic rodent genotoxicity assays with the *lacI* or *lacZ* genes, *gpt* delta rodents can detect the genotoxicity of radiation and cross-linking agents that induce DNA strand breaks. I will introduce the basic features of *gpt* delta TG assay and discuss the future direction of battery of in vivo and in vitro assays in genotoxicology.
Reference: T. Nohmi, K. Masumura, Molecular nature of intra-chromosomal deletions and base substitutions induced by environmental mutagens, Env. Mol. Mutagen., 45, 150-161, 2005

1670 EXPLORING THE LINK BETWEEN HIF-1 AND RADIOPROTECTION.

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The search for potential radioprotective compounds has recently escalated in light of the 9/11 attacks. There is mounting evidence that HIF-1, a transcription factor and the master regulator of hypoxia, may have a role in radioprotection. Our investigation focused on small molecules, which stabilize HIF-1α, one of the two subunits of HIF-1. The immune system is most sensitive to acute radiation damage. Therefore we examined the radioprotective effects of cobalt chloride (CoCl2), dimethyloxalylglycine (DMOG), dibenzoylmethane (DBM) and hydralazine in two immune system cell lines, Til1 and DC2.4. Both DMOG and hydralazine upregulated HIF-1 target genes in Til1 cells and confered radioprotection, as measured by a proliferative assay. Furthermore, CoCl2, DMOG, DBM and hydralazine all upregulated HIF-1 target genes in DC2.4 cells and also confered radioprotection against ionizing radiation. In order to assess the activity of these compounds in vivo, mice were treated with each compound for 24 and 48 hours, using gavage or intraperitoneal (IP) administration. We used the spleen for analysis of HIF-1 target genes since this organ is a collection of immune system cells. IP administration of DBM and ethyl-3,4-dihydroxybenzoate (EDHB), another HIF-1α stabilizer, both upregulated HIF-1 target genes in the mouse spleen as confirmed by immunohistochemistry. We are now testing whether these treatments protect the spleen and whole mouse against radiation toxicity.

1671 REPAIR OF RADIATION INDUCED DNA STRAND BREAKS IN P53 DEFICIENT AND WILD-TYPE MOUSE HEMATOPOIETIC STEM CELLS IS STRAIN DEPENDENT.

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Loss of heterozygosity (LOH) is caused by misrepair of carcinogen induced DNA damage that results in the loss of tumor suppressor genes and dysregulation of signal pathways that suppress cancer. Coordination of DNA strand break repair pathways is dependent on the phase of the cell cycle, DNA replication, and expression of the p53 tumor suppressor protein. We have reported that carcinogens (ionizing radiation, benzene, melphalan, etc.) rapidly induce tumors with the LOH of the wild type Trp53 allele in p53 heterozygous mice carrying an inherited p53 null mutation and a functioning wildtype allele. The LOH phenotype observed in ionizing radiation (IR) induced tumors is highly penetrant in the C57BL/6 (B6) mouse strain and appears low in the DBA/2 (D2) strain. IR induced Trp53 sequence loss is the greatest in B6>B6C3F1>C3H>B6D2F1>D2. D2 alleles suppress both LOH and tumor rates. Using primary cultures of HSC, B6 and D2 isogenic mouse HSC, we have observed significant differences in the abundance of DNA repair gene transcripts up to 3 h post-irradiation. Terminal deoxynucleotidyl transferase (TdT) assay for quantification of DNA strand breaks and the time required to resolve breaks is significantly different between these two strains. The data suggest that misrepair of strand breaks and LOH is a quantitative trait (polygenic) dependent upon the DNA damage repair capacity. Analysis of NHEJ repair gene haplotypes by similarity matrices indicates that allelic diversity across isogenic strains in some genes is significant but many components of this repair pathway are identical by descent. In order to identify the allelic variants (haplotypes) of genes associated with this strand break repair and LOH phenotype additional phenotyping and functional analysis of multiple isogenic strains are required. Determination of the allelic variants of genes causally related to DNA damage and repair with altered function is critical in order to understand the differences in risk due to exposure to environmental mutagens.

1672 THE HPRT/SR ASSAY: A NOVEL METHOD TO ASSESS STRESS RESPONSE PATHWAY ALTERATIONS IN CANCER.

D. M. Walker and R. J. Albertini. *BioMosaics, Burlington, VT.* Sponsor: V. Walker.

Prokaryotic stress responses involve survival mechanisms that include cytoprotective processes; if these fail, stressed cells can initiate cellular mutational mechanisms with high mutant frequencies to alter their genomes and thereby increase the chances of survival for a subset of cells. Indirect evidence suggests that similar stress-associated mutational mechanisms, leading to a mutator phenotype, occur in human cells and are involved in early stages of cancer development. We have developed a novel in vitro epithelial cell-based assay (the HPRT/SR assay) to investigate molecular changes associated with induction of mutational cell response mechanisms in a stress environment, induction of genomic instability, and their relationship to tumorigenicity. The assay consists of several steps including exposure of cells, growth without selection to allow for phenotypic expression, and selection with an agent that induces a genotoxic stress response. The results support the hypotheses that (1) stress-associated hypermutational mechanisms similar to those described for bacteria occur in mammalian cells, (2) pre-exposure to a low dose of a carcinogen predisposes a subset of cells to express these mutational mechanisms, (3) mammalian epithelial cells have an unexpected capacity for an error-free survival response to genotoxic stress, and (4) this cytoprotective response can occur in the absence of the hypermutation response. Cells expressing the hypermutation response display a mutator and drug-resistant phenotype. A review of the literature supports the conclusion that the relationships between pre-exposure to a mutagen or carcinogen, alterations in stress response gene regulons and progression towards and/or expression of the tumorigenic phenotype have not been evaluated previously in a single assay, supporting the potential for the HPRT/SR assay to provide novel information about molecular events associated with cancer induction.

1673 GENOMIC ARCHITECTURE AND INSTABILITY OF HUMAN RIBOSOMAL RNA GENE CLUSTERS.

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In humans, the genes responsible for producing the structural RNA components of the ribosome are arranged as clusters of repeated units distributed across twelve chromosomal loci. The extreme sequence identity, size, and tandem sequential arrangement make these gene clusters optimal substrates for non-allelelic homologous recombination (NAHR). The structure of these loci and their susceptibility to NAHR-mediated rearrangement was largely unknown. In order to formally determine the extent of human variation and the degree of instability in ribosomal RNA genes (rDNA), we isolated genomic DNA from white blood cells of healthy human volunteers, and used restriction enzyme digestion, pulsed-field gel electrophoresis, and in-gel-hybridization Southern blotting to produce an electrophoretic rDNA karyotype for each individual. The cluster lengths exhibit striking variability between and within individuals, manifesting essentially complete heterozygosity. Our analysis of multi-generational families demonstrates the rDNA clusters are subject to meitoic rearrangement at a frequency greater than 10% per cluster, per meiosis. Using this assay, we were also able to detect somatic recombination as evidenced by mosiacism in the lengths of the rDNA clusters in some individuals. We can detect these changes when as few as 2% of the total cell population contains deviant cluster lengths. Because this assay detects physical alterations in genomic architecture, we believe it will be a valuable means of tracking structural changes that arise due to increased recombination in malignant versus normal cell populations, and also may be useful as a sensitive measure of mutagenic potential.

1674 FORMALDEHYDE INDUCES TOXICITY *VIA* A P53-DEPENDENT SIGNALING PATHWAY.

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Formaldehyde is a widely used chemical that is known to cause the formation of numerous DNA-protein crosslinks. The mechanism by which these lesions cause toxicity is currently unknown. In this work, we examined the role of p53 in formaldehyde-induced toxicity. All experiments were done using lung epithelial H460 cells. This cell line was chosen due to the fact that formaldehyde exposure occurs most commonly by route of inhalation. H460 cells stably expressing p53-targeting siRNA were validated for decreased p53 expression, and used as an effective p53 knockdown model in these experiments. We found that in p53-proficient cells (wild-type H460), formaldehyde caused a strong induction of p53 protein associated with its phosphorylation at Serine 15. In addition, we observed an upregulation of p21, a downstream target of p53 that is known to play a major role in cell cycle arrest. Accordingly, H460 cells proficient in p53 displayed a significant cell cycle arrest that was much less pronounced in p53 knockdown cells. Clonogenic experiments detected an increased survival of p53 knockdown cells when compared to p53-proficient cells. We have also shown that formaldehyde-induced apoptosis was p53-dependent. The induction of apoptotic cells was established by the appearance of subdiploid cells and PARP cleavage. In conclusion, our findings provide evidence that a p53-dependent signaling pathway is highly responsive to the presence of DNA-protein crosslinks and plays a key role in formaldehyde-induced cell cycle changes and other toxic responses.

1675 TOXIC ACTIVITIES IN WOOD DUST FROM RED OAK.

M. Wilson, R. Rando and C. A. Miller. *Environmental Health Sciences, Tulane University, New Orleans, LA.*

Wood dust is a complex environmental agent that is associated with numerous adverse health effects, including cancer. In particular, professional woodworkers have an elevated risk of developing cancer of the upper respiratory (nasal) tract. Few molecular and mechanistic studies have addressed the toxic components in wood dusts, so we have begun to characterize this occupational carcinogen. Since red oak is commonly used for the manufacturing of furniture and cabinets, we studied the toxic activities of this wood dust. Red oak dust, and methanol extracts from this dust, reduced growth and killed hamster fibroblast cells grown in vitro. The TD50 of the methanol extract of wood dust from red oak was approximately 200 ug/ml for inhibition of colony formation. Following treatment with the extracts, the surviving cell fraction displayed a dose-related increase in large multinucleated cells. Methanol extracts from red oak dust showed dose-dependent activation of human aryl hydrocarbon receptor signaling in a recombinant yeast bioassay. Agents known to activate aryl hydrocarbon receptor signaling are often toxic, proinflammatory, and carcinogenic. Collectively, our data suggest that red oak dust, perhaps acting in conjunction with other agents present in woodworking operations, contains a compound(s) that could contribute to carcinogenic processes.

1676 ZIDOVUDINE INDUCES APOPTOSIS, DELAYS CELL CYCLE PROGRESSION AND DECREASES TELOMERASE ACTIVITY IN HUMAN HEPATOMA HEPG2 CELLS.

J. Fang and F. A. Beland. *NCTR, Jefferson, AR.* Sponsor: T. Chen.

Zidovudine (3'-azido-3'-deoxythymidine; AZT) is used extensively for the treatment of AIDS and the prevention of mother-to-child transmission of HIV-I. AZT is a DNA replication chain terminator and capable of inducing various types of genotoxicity. The present study examined the effects of AZT on apoptosis, cell cycle progression, and telomerase activity. Human hepatoma HepG2 cells were incubated with 0 – 100 μM AZT for up to 4 weeks. The number of viable cells was decreased by AZT, in a dose-dependent manner, at exposures > 48 h. After a 1-week post-AZT treatment period (PR5), following 4 weeks of AZT, the cell viability of AZT-treated cells was 17 – 93% of control cells. Necrotic cell death was not associated with the decrease in viable cells; however, there was an induction of apoptosis, which occurred mainly at the two highest doses of AZT and after 48 h. A significant decrease in telomerase activity was observed with 100 μM AZT, from 48 h to 4 weeks, and with 20 μM AZT, from 3 to 4 weeks. Apoptosis and telomerase activity returned to control levels upon removal of the AZT (PR5). AZT produced a dose-dependent accumulation of cells in the S and G2/M phase, with a corresponding depletion of cells in the G1/G0 phase. The disruption of cell cycle progression persisted in AZT-treated cells after removal of the AZT (PR5). In AZT-treated cells, the protein levels of checkpoint 1 (chk1), checkpoint 2 (chk2), and the cyclin dependent kinase Cdc2 p34 were significantly decreased at all time points, whereas cyclin A was significantly increased from 24 to 48 h. The tyrosine-specific kinase Wee1, the signalling intermediate 14-3-3b, and proliferating cell nuclear antigen were virtually unchanged. Interestingly, the levels of phosphorylated chk1 (Ser345) and chk2 (Thr68) were significantly increased. These data indicate that the combination of an increase in apoptosis, a delay of cell cycle progression, and decrease in telomerase activity is contributing to the loss of viable cells with AZT treatment, and that chk1 and chk2 may play an important role in the delay of cell cycle progression.

1677 CENTROSOMAL AMPLIFICATION INDUCED BY ANTIRETROVIRALS COMMONLY USED IN THE THERAPY OF HIV.

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In cultured cells, exposure to the highly-effective nucleoside reverse transcriptase inhibitor (NRTI) Zidovudine (AZT) induces genomic instability, causing cell cycle arrest, micronuclei, sister chromatid exchanges, and shortened telomeres. In previous studies, we demonstrated for the first time that AZT induced centrosomal amplification, (> 2 centrosomes /cell), a novel manifestation of genotoxicity. Chinese Hamster Ovary (CHO) cells exposed for 24 hours to 0, 10 and 100 μM AZT produced 0.27, 0.66, and 1.13% of cells with > 2 centrosomes, respectively. Here, centrosomal amplification was explored in CHO cells exposed for 24 hr to other commonly used NRTIs by staining with an anti-pericentrin antibody. A dose response curve using 5 doses of each NRTI as well as thymidine, and a time response curve at 24, 48 and 72 hr were performed first to determine cytotoxicity for each individual compound. The agents and doses used for the pericentrin analysis were as follows: Lamivudine (3TC) 12 and 122 μM, Didanosine (ddI) 10 and 102 μM, and Stavudine (d4T) 8.8 and 89 μM, and approximately 1,000 cells/group were scored under fluorescence microscopy. At 24 hours, cell survival ranged from 74 to 89% for 3TC, 86 to 92% for ddI, and 80 to 92% for d4T and 100% for thymidine. A dose-dependent increase in centrosomal amplification was observed for all the NRTIs studied. For 3TC, 0.29, 0.98, and 1.26 % of cells exposed to 0, 12, or 122 μM 3TC, respectively, contained > 2 centrosomes. For ddI 0.42, 0.97, and 1.38% of cells exposed to 0, 10, or 102 μM ddI contained >2 centrosomes. In addition, centrosomal amplification occurred in 0.29, 1.0, and 1.11 % of cells exposed to 0, 8.9, and 89 μM d4T, respectively. The high dose for each compound and the low dose for AZT and ddI were significantly different ($P < 0.005$) compared to the unexposed control. Therefore, centrosomal amplification is not limited to AZT but also occurs with other commonly used nucleoside analogs in the following order of increasing toxicity: d4T < AZT < 3TC < ddI.

1678 THE END DRAWS NEAR: TELOMERE SHORTENING INDUCED BY A QUINONE METABOLITE OF PCB3.

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Recent studies have reported surprisingly high levels of mono and di-chlorinated biphenyls in several indoor and outdoor samples. One of these semivolatile PCB congeners is 4-chlorobiphenyl (PCB3), a major component of one commercial

PCB Aroclor mixture and indoor air samples. PCB3 is more easily converted to hydroxylated metabolites than higher chlorinated congeners, which makes metabolites of PCB3 particularly interesting for toxicological research. Previous studies have shown that PCB3 induces point mutations and its metabolites increase oxidative stress and form covalent adducts with DNA. Telomeres, which protect the ends of chromosomes, play an important role in carcinogenesis and senescence. They are damaged by oxidative stress. Our working hypothesis is that PCB3-quinone metabolites are the/an ultimate carcinogen by producing oxidative stress-related damage to the telomeres. Human keratinocytes (HaCaT cells) and primary fibroblasts were treated continuously for 12 weeks with 2-(4'-chlorophenyl)-1,4-benzoquinone (PCB3pQ). Telomere length was determined using a quantitative polymerase chain reaction (qPCR) method. Telomeric DNA levels decreased approximately 20% in HaCaT cells treated with PCB3pQ at 5 μM compared to solvent control-treated cells. The telomere signal of primary fibroblasts also shows a decreasing trend with PCB3pQ treatment, but this effect was not statistically significant and confounded by differences in cell doublings during the 12 week period. These preliminary results urge further mechanistic studies concerning the effects of PCB congeners and metabolites on telomeres. (Supported by NIEHS P42 ES013661 and an Iowa CHEEC Seed Grant)

1679 GENOTOXICITY OF PCB3 METABOLITES *IN VITRO* – WHICH ONE IS THE BAD GUY?

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Hot summer days in Chicago, classroom building from the 70s or neighborhoods downwind from Superfund sites – they all may expose us to elevated levels of lower chlorinated PCBs. PCB3 (4-monochlorobiphenyl), a member of this group of semivolatile PCB congeners, is readily metabolized to monohydroxy- and dihydroxy-biphenyls (2,5-hydroquinone, 3,4-catechol), and further to quinones (PCB3-2,5-pQ and PCB3-3,4-oQ). Recent studies demonstrated that PCB3 has an initiating potential in liver of male rats and induces mutations in the livers of transgenic Fisher 344 (BigBlue®) rats. Our question was what type of genotoxic damage might be involved and which metabolite(s) is/are responsible for these effects. We already showed that only the quinoid metabolites of PCB3 induce gene mutations in cultured Chinese hamster V79 cells. Here we measured the effects of PCB3 and its dihydroxy- and quinone metabolites on sister chromatid exchange frequency and cell cycle progression in cultured V79 cells. SCE are coordinated reciprocal single strand break-and-rejoining events during the S-phase. Only the PCB3-3,4-Cat significantly increased SCE levels. PCB3-2,5-HQ doubled the chromosome number (4n) in >90% of all metaphases after exposure for 2 cell cycles. Surprisingly these mitosis were not harlekin stained, excluding failed cell division as mechanism. Instead a delay in the cell cycle progression, cell fusion and later a reduction of chromosome number were observed. Since nearly all cancer cells are hyperdiploid, polyploidization and uneven chromosome loss is hypothesized as one possible mechanism of carcinogenesis. Also, the ability to induce SCE and cell transformation in vitro correlates surprisingly well. Thus different PCB3 metabolites may be involved in cancer initiation by different mechanisms, i.e. gene mutation (quinones), SCE (3,4-catechol) or polyploidization (2,5-hydroquinone). More studies are needed to understand the importance of each mechanism for PCB3's potential as carcinogen in vivo. (Supported by NIEHS P42 ES013661 and DOD DAMD17-02-1-0241)

1680 INVESTIGATING GAMMA-H2AX EXPRESSION AFTER ACUTE AND *IN UTERO* BENZENE EXPOSURE.

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Benzene is an ubiquitous pollutant and industrial solvent that has been identified as a clastogen and human leukemogen. Early exposure to carcinogens such as benzene has been suggested to play a role in the development of childhood leukemia, however the association remains controversial. Clastogenic agents such as benzene can cause an increase in the frequency of DNA double-stranded breaks (DSBs), which results in the initiation of DNA repair mechanisms. Erroneous repair of DSBs is one of the mechanisms that can initiate cancer. One of the early events in DSB repair is the phosphorylation of the histone H2AX (which is then referred to as gamma-H2AX), which precedes the recruitment of other repair proteins including the MRN repair complex, Rad50, and BRCA1. Since the phosphorylation of H2AX occurs rapidly after the induction of DSBs, gamma-H2AX is widely used as a marker for DSB formation and repair. To investigate the hypothesis that benzene exerts toxicity through the induction of DSBs, male C57Bl/6 mice were treated with either 400mg/kg benzene or vehicle via an i.p. injection and sacrificed 1, 3, 6, and 24 hours after exposure. Liver and bone marrow samples were collected and immunoblotting techniques were used to detect changes in gamma-H2AX levels correlating to DSB events. Preliminary data suggests that benzene increases levels of gamma-H2AX. In order to determine whether in utero exposure to benzene can result in fetal DNA damage, ongoing studies involve C57Bl/6 dams treated with either benzene or vehicle during gestation and fetal livers are examined at gestational day 16 for increases in gamma-H2AX.

1681 MUTAGENIC EVALUATION OF THE AZO DYE DISPERSE ORANGE-I AND DETERMINATION OF THE MECHANISM OF DNA DAMAGE.

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The azo dyes are the most important class for dyeing process of textile industry and the Disperse Orange I has been largely used, but little is known about its toxic potential. The aim of this work was to evaluate the cytotoxicity and mutagenicity of the Disperse Orange I, using different endpoints, as well as the study of the possible mutagenic mechanism. For the Salmonella mutagenicity assay we used TA98 and YG1041 strains, with and without metabolic activation, with doses varying between 0.1 to 5 mg/plate. We also used the micronucleus cytokinesis-block in lymphocytes assay using fresh blood cultivated in RPMI medium with phytohemaglutin. For this test, the dye concentrations were 25 to 100 mg/L. The cytotoxicity was evaluated in HepG2 cells by the MTT assay. The mutagenic mechanism study was based on the formation of stable radical of the dye generated by spectroelectrochemical oxidation and its interaction with guanosine (nitrogenated base of DNA). The radical generation was evaluated by electrochemical analysis and alterations on the UV-Vis spectra profile using a diode array spectrophotometer. Our results showed that the dye is cytotoxic for HepG2 cells and induces the micronucleus formation. With the Salmonella assay, we observed that the Disperse Orange I is mutagenic with the strain YG1041, that overproduces nitroreductase and o-acetiltransferase. In the presence of S9, the dye has a light increase of the mutagenicity with the same strain. The spectroelectrochemical analysis showed that Disperse Orange (with typical band at 450 nm) generated a stable radical when oxidized at +0.90V, detected by an extra band at 670 nm, which is 100% suppressed in the presence of guanosine. We concluded that the orange dye has mutagenic and cytotoxic effects and its metabolization, mainly by oxidation reactions, is important for its toxic effect that is related to the formation of a stable radical with DNA after an oxidation step.

1682 THE GENOTOXICITY AND CARCINOGENICITY OF SULFONATED AZO-DYES: A STRUCTURE-ACTIVITY EVALUATION.

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A number of azo-dyes are mutagens and rodent carcinogens; however, there is a subset that is neither genotoxic nor carcinogenic. Assessment of azo-dyes requires evaluation of the parent dye, azo-reduction products, and metabolites. The Salmonella protocol must include conditions to ensure azo-reduction. A previous analysis of azo-dyes concluded that dyes with an aminosulfonic acid functional group were neither mutagenic in Salmonella nor carcinogenic. It is unclear if azo-reduction was considered in the evaluation; the Salmonella assay would not have fully assessed mutagenicity if azo-reduction conditions were not used. Since azo-reduction is critical to the predictivity of the Salmonella assay, the data set was re-evaluated and updated to include genotoxicity and carcinogenicity data for additional sulfonated azo-dyes. The data were also supplemented with Salmonella data alone for 25 sulfonated azo-dyes. Another consideration is whether the aminosulfonic group is present on all azo-reduction products. The previous analysis considered dyes containing at least one azo-reduction product with an aminosulfonic acid functional group. This analysis considered only dyes with the aminosulfonic acid functionality on all azo-reduction products. Of 20 dyes in the original analysis, 2 were rejected since carcinogenicity data was not found and 7 were removed since all azo-reduction products did not contain an aminosulfonic acid functionality. The Salmonella and carcinogenicity data for the remaining azo-dyes and data for additional dyes were reviewed to determine if the Salmonella assay included azo-reduction conditions; and, that the rodent bioassays adequately assessed carcinogenic potential. Analysis of the current data set revealed the lack of mutagenicity in the Salmonella assay does correlate with lack of carcinogenicity. Based on this, it can be concluded that the Salmonella assay incorporating azo-reductive conditions is predictive of carcinogenic potential for azo-dyes containing aminosulfonic acid functionality on all azo-reduction products.

1683 EFFECTS OF SIDE STREAM TOBACCO SMOKE ON MICE DEFICIENT IN THE REPAIR OF OXIDATIVE DNA DAMAGE.

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Side stream tobacco smoke (SSTS), the main component of secondhand smoke, is classified as a human carcinogen, but has been difficult to study in research animals. Cigarette smoke causes free radicals and inflammation, both leading to DNA damage. Thus, animals lacking functions that repair oxidative DNA damage, with the propensity to develop spontaneous lung tumors, may be more sensitive to the biological activity of SSTS. Genome rearrangements including DNA deletions are involved in carcinogenesis and an *in vivo* assay for DNA deletions (pink-eyed unstable, p^{un} reversions) is inducible by genetic as well as environmental cancer predisposing factors. P^{un} mice are a convenient model because they allow for a visual assay examining the frequency of DNA deletion events. We use mice lacking the gene *Ogg1* or *Myh*, or both which are involved in base excision repair, removing oxidatively damaged DNA bases. We exposed mice lacking Myh, Ogg1, or both, with a pink-eyed unstable ($P^{un}P^{un}$) background, to SSTS. Our preliminary studies show that Myh-deficient mice are more sensitive to SSTS than their wildtype littermates when exposed to 1.6 mg/m^3 total particulate matter for 10 days. Thus humans with polymorphisms in the *MYH* gene may be more susceptible to SSTS-induced DNA damage. The application of animals deficient in DNA repair enzymes can provide insight into mechanisms used in wildtype animals to defend or protect against toxicity and conversely, be used to look at genetic predisposition to toxicity or insult. Understanding gene-environment interactions in SSTS as well as the interactions of oxidative stress and DNA damage will aid in the development of chemoprevention and treatment of lung cancer.

1684 EFFECT OF CIGARETTE FILTER VENTILATION ON CYTOTOXICITY, MUTAGENICITY, INFLAMMATION AND FREE RADICALS OF SMOKE PARTICULATE MATTER.

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Filter ventilation is a construction method used to decrease the yield of mainstream cigarette smoke. Some Ames mutagenicity studies have shown that cigarette smoke wet total particulate matter (WTPM) collected from ventilated cigarettes has slightly higher levels of specific activity (revertants per unit mass of mainstream smoke particulate matter) than condensates from unventilated cigarettes. In this study, mutagenicity, cytotoxicity and IL-8 induction were determined for Industry Monitor IM16 cigarettes which were laser-ventilated at 15%, 30% and 60% filter ventilation. Cigarettes were smoked under standard FTC conditions (2 sec draw, 1 min puff interval, 35 ml puff volume) and WTPM was collected on Cambridge filter pads and extracted in DMSO. Collected WTPM and water yields per cigarette decreased with increasing filter ventilation. WTPM tested in the Ames (preincubation) assay using Salmonella strains TA98 and TA100 under S9+ conditions resulted in statistically significant increases in specific activity (revertants/μg WTPM) with increasing filter ventilation, in TA98 only ($p < 0.05$). Results in both strains for Dry Particulate Matter (WTPM minus water) and "tar" (WTPM minus water and nicotine) were similar to WTPM, suggesting the observed differences in Ames activity were not due to differences in the WTPM water content. Neutral Red Uptake, measured in A549 cells, resulted in no observed cytotoxicity differences between samples ($p > 0.05$). IL-8 release from A549 cells increased with increasing filter ventilation; however, the differences were not statistically significant ($p > 0.05$). Cigarette smoke WTPM radicals, measured using Electron Spin Resonance (ESR), did not significantly change in the number of spins / mg WTPM; however, the number of spins / cigarette decreased with increasing filter ventilation due to decreasing tar delivery with increasing ventilation. This difference is due to the decreasing WTPM deliveries with increasing filter ventilation.

1685 DETERMINANTS OF ASBESTOS FIBER-INDUCED GENOTOXICITY.

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Asbestos is a family of naturally occurring mineral fibers associated with the development of various diseases. Adverse effects of asbestos are evident at the molecular and cellular levels (DNA damage, lipid peroxidation, etc.) as well as at the tissue and whole organism level (fibrosis, cancer, etc.). Although in early analyses asbestos fibers were negative or only marginally positive by the Ames test, current data confirm fiber genotoxicity. Literature review identified two main mechanisms that may contribute to fiber-induced genotoxicity: DNA damage resulting from reactive oxygen species (ROS) production, and physical interference with mitosis leading to clastogenicity. The analysis described here focuses on the role of fiber size, components and biopersistence in the genotoxic response. Fiber length may be a key determinant of relative genotoxicity, since longer fibers cannot be removed efficiently and induce prolonged inflammation and reactive oxygen and nitrogen species (ROS/RNS) generation. Mechanisms such as receptor binding, cell signaling and ROS/RNS production may also be influenced by fiber dimension and mineralogy. Variability in the extent of DNA damage among fiber types may be influenced by metal components of the fibers (e.g. chrysotile vs. amosite). For example, iron and other transition metals in asbestos fibers increase ROS production, and result in a corresponding increase in DNA damage. Fiber length and diameter also influence the ability to disrupt the mitotic process. This analysis of the literature provides a better understanding of the basis of fiber-induced genotoxicity by examining mechanisms with respect to the fiber characteristics (dimension, mineralogy and morphology) that influence the genotoxic response. Through this understanding of the influence of determinants of fiber-induced genotoxicity we can better inform uncertainty in risk assessment of asbestos exposure. Disclaimer: The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the US EPA.

1686 CHEMICAL SYNTHESIS AND IDENTIFICATION OF N3-ADENINE ADDUCTS FORMED BY 1- OR 2-BROMOPROPANE WITH CALF-THYMUS DNA AT THE PHYSIOLOGICAL CONDITION.

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2-Bromopropane (2-BP) has been used as a replacement for chlorofluorocarbon and 1,1,1-trichloroethane as a cleansing solvent in the electronic industry. But 2-BP was found to cause amenorrhea in female and oligospermia in male workers when exposed to it. Due to the toxicity of 2-BP, it has been increased tendency to use 1-bromopropane (1-BP) as an alternating cleansing solvent to 2-BP. However, 1-BP has also been reported to be neurotoxic in rats. Therefore, in order to elucidate mechanism of 1-BP and 2-BP induced toxicities in the molecular level, formation of N3-adenine adducts by 1-BP and 2-BP was investigated at the physiological condition. N3-adenine adducts of 1-BP and 2-BP were chemically synthesized and structurally characterized by 1H NMR, 13C NMR, UV, HPLC and ESI LC/MS/MS to utilize as a reference materials. N3-Adenine adducts were detected and identified by UV, HPLC and ESI LC/MS/MS after incubation of calf-thymus DNA with 1-BP and 2-BP at the physiological condition by ESI LC/MS/MS. This result might explain the toxic effect of 1-BP and 2-BP by the formation of N3-adenine adducts.

1687 AN EVALUATION OF THE P53 KNOCKOUT MOUSE IN THE EARLY DETECTION OF THE CARCINOGENIC POTENTIAL OF N-METHYL-N-NITROSUREA.

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P53 is a tumour suppressor gene. Loss of one or both alleles drives tumourogenesis (Macleod et al J Pathol 187,43-60 1999). It has therefore been proposed that mice having one or both alleles deleted will develop chemically induced tumours at a shorter time after exposure when compared to wild type animals, although studies of up to 26 weeks duration have failed to demonstrate this unequivocally. In order to study a longer evaluation period after exposure, N-methyl-N-nitrosourea (MNU) a potent carcinogen in different species of laboratory animal and in a number of organ systems (Terracini et al Br. J Cancer 24,588-598 1976) was dosed on a single occasion to TSG-p53® (C57BL/N5-T) heterozygote mice and the outcome evaluated for up to 39 weeks. Groups of 25 male and 25 female mice were administered a single dose of N-methyl-N-nitrosourea (MNU), by oral gavage at 90 mg/kg or by intraperitoneial (ip) injection at 30 mg/kg for up to 39 weeks. A further control group received the vehicle alone by oral gavage.

After a single gavage dose of 90mg/kg MNU 12/25 males and 24/25 females failed to survive the evaluation period. After 30mg/kg ip 13/25 females failed to survive. The predominant cause of death was thymic masses (malignant lymphoma). The majority of tumours in the female 30 mg/kg ip dosed animals developed after 26 weeks, whilst in the animals dosed with 90 mg MNU by gavage they developed before 26 weeks. Malignant lymphoma was recorded in 9/25 males, 22/25 females 90 mg MNU/kg and 0/25 males and 6/25 females at 30 mg MNU/kg. Uterine adenocarcinomas were observed in 5/25 oral dose females and 8/25 intraperitoneal dose females. All of these tumour incidences were higher than the control group incidence where survival was unaffected by treatment.This model appeared to produce a clear positive effect for a single oral dose of a potent carcinogen within 26 weeks after exposure, but not for the intraperitoneal route, where a clear effect was only seen after 39 weeks in females.

1688 DETECTION OF PIG-A MUTANT CELLS IN PERIPHERAL BLOOD OF MACACA MULATTA.

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We have investigated the use of peripheral blood from the non-human primate (NHP) rhesus monkey (Macaca mulatta) as a model system for mutation detection. The rhesus monkey is metabolically closer to humans than most common mammalian laboratory animals, and may be a relevant model for hazard identification and risk assessment. To validate the model, conditions were determined for in vitro selection and expansion of proaerolysin-resistant Pig-A mutant lymphocytes from peripheral blood obtained by routine venipuncture. Pig-A mutant lymphocytes were enumerated in the peripheral blood of several ~3-year-old male monkeys receiving daily doses of the electrolyte replenisher Prang™ (a common carrier for oral delivery of drugs in NHPs), and in the blood of one ~3-year-old male monkey treated with a single i.p. injection of 77mg/kg ENU at ~2 years of age. In addition, lymphocyte HPRT mutant frequency (MF) was measured in all of these animals. The spontaneous Pig-A and HPRT MFs were low in animals receiving Prang (0 to 4×10E-6). The acute treatment with ENU resulted in a clearly detectable increase in Pig-A and HPRT MFs (up to ~28×10E-6 and ~30×10E-6, respectively). Currently, we are developing flow cytometry conditions for detecting erythrocytes stemmed from Pig-A mutant bone marrow precursors. The mutant erythrocytes will be identified by the deficiency of cell surface markers attached to the cellular membrane via glycosylphosphatidyl inositol (GPI) anchors. A potential increase in the frequency of GPI-deficient erythrocytes in the ENU-treated animal will be investigated. Our preliminary results indicate that rhesus monkey can be a valuable model for the identification of agents that may impact upon human health as mutagens and that the Pig-A gene can be a sensitive target for detection of mutation in white and red blood cells.
This study was partially funded by the NICHD Best Pharmaceuticals for Children Act of 2002. The views presented in this article do not necessarily reflect those of the Food and Drug Administration.

1689 DETECTION AND QUANTITATION OF 1, N^2-3-METHYLINDOLEDEOXYGUANOSINE ADDUCTS AND 1, N^6-3-METHYLINDOLEDEOXYADENOSINE ADDUCTS IN HUMAN LUNG EPITHLIAL CELLS BY LC/MS.

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3-Methylindole (3MI) is a chemical in cigarette smoke and a byproduct of the catabolism of tryptophan in the intestinal tract. 3MI is considered to be selective pneumotoxicant. 3MI's pneumotoxicity has been attributed to bioactivation of 3MI to 3-methyleneindolenine by cytochrome P450 2F enzymes (CYP2Fs), and this reactive intermediate alkylates DNA. Here we describe a sensitive and specific liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) method to detect and quantify 3MI-DNA adducts from lung cells. Incubation of 3MI with calf thymus DNA in presence of CYP2F3 produced 1,N^2-3-methylindoledeoxyguanosine (3MI-dG) and 1,N^6-3-methylindoledeoxyadenosine (3MI-dA). These adducts were not detected in the control samples in the absence of NADPH. Treatment of human lung bronchial epithelial cells (BEAS-2B) overexpressing CYP2F1 with 3MI (200 μM, 2 hours, or 1 μM, 24 hours) produced DNA adducts, 3MI-dA adducts approximately 10 adducts/10^8 DNA bases and 4 adducts/10^8 DNA bases, and for 3MI-dG approximately 4 adducts/10^8 DNA bases and 2 adducts/10^8 DNA bases, respectively. Treatment with 1 μM 3MI for 24 hours was not toxic to the cells whereas 200 μM was toxic for the same period of time. Our results demonstrated that 3MI-DNA adducts can be quantified at very small amounts from human lung cells. This is the first example of the detection of 3MI-DNA adducts in lung epithelial cells at a concentration/time period that is not toxic. This method will facilitate future studies to differentiate threshold levels of DNA adducts between toxic and mutagenic processes. This research was supported by NIH grant # HL13645 from the national Heart, Lung, and Blood Institute.

IS 1690 MENTORING 101 - HOW TO MENTOR, HOW TO BE MENTORED.

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A mentor is a teacher or trusted counselor who may play a large role in how satisfied a student or colleague is with his/her studies or career. Some individuals are natural mentors and excel at this activity. However, for many, mentoring skills need to be developed. This session will explore this issue and address such questions as: What makes a successful mentor? How does a mentor exhibit the empathy, understanding and trustworthiness required to form a positive connection? How does a mentor acquire those characteristics? Does a mentor know how to listen to goals, help the mentee to clarify and understand them and provide appropriate resources or contacts to facilitate career advancement? This session is intended for both those scientists seeking to improve their mentoring skills and those who want to learn more about the potential benefits of receiving mentoring. The ideas fostered through this session will positively impact the expanding mentoring activity of the Society by providing basic advice on mentoring relationships.

1691 ETHANOL INHIBITS CARBACHOL-INDUCED AXON OUTGROWTH IN HIPPOCAMPAL NEURONS BY INHIBITING ERK1/2.

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In utero alcohol exposure can lead to fetal alcohol syndrome, which is characterized by facial dysmorphology, growth deficiencies, and CNS abnormalities, including altered neuronal development. The cholinergic agonist carbachol stimulates axonal growth through the activation of M1 muscarinic receptors in prenatal rat hippocampal pyramidal neurons, and this effect is inhibited by ethanol (20-75 mM). The goals of this study were to identify the intracellular pathway activated by carbachol in neurons and responsible for its neuritogenic effect, and to investigate the effect of ethanol on carbachol-activated signaling molecules. Hippocampal neurons were treated with specific inhibitors in the presence of carbachol and axon growth was measured by morphometric analysis. We found that the Ca2+ chelator BAPTA-AM, the PKC inhibitors GF 109203X, PKCβ inhibitor, Ro-32-0432, and Gö9676, and the ERK1/2 inhibitors PD98059 and U0126 blocked carbachol-induced axonal outgrowth, suggesting the involvement of a pathway traditionally activated by Gq/11. By contrast, inhibitors of another pathway activated by carbachol in astrocytes involving PLD, PKCζ, p70S6K, and NF-κB activation did not affect axon outgrowth. Carbachol increased the levels of intracellular Ca2+ and caused ERK1/2 phosphorylation. Ethanol inhibited the increase in intracellular Ca2+ only at 75 mM, while ERK1/2 phosphorylation was inhibited starting at 50 mM. These findings suggest that the inhibitory effect of ethanol on muscarinic receptor-induced hippocampal neuron differentiation may occur through the inhibition of carbachol-activated ERK1/2 pathway.
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1692 MODERATE MATERNAL IRON INADEQUACY WORSENS NEUROBEHAVIORAL OUTCOMES IN A RAT MODEL OF DEVELOPMENTAL ETHANOL EXPOSURE.

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Prenatal alcohol exposure causes behavioral and cognitive disorders collectively known as fetal alcohol spectrum disorders (FASDs). Prevention of alcohol use is difficult, thus therapies that could ameliorate alcohol's damage are desirable. FASD severity increases with parity, implying the depletion of a protective maternal factor, perhaps a nutrient. Iron deficiency (ID) is the most common nutritional deficiency in women and causes neurodevelopmental deficits that parallel those of FASD. We hypothesized that moderate maternal ID may exacerbate alcohol's effects upon the developing brain. We tested this hypothesis in ID or iron sufficient rat pups exposed to alcohol during the brain growth spurt period (postnatal days 4-9; P4-9). ID dams were mildly ID on P5 and were anemic by weaning, with increased red-cell distribution width and decreased liver iron and mean corpuscular hemoglobin, an iron profile that mimics that of many pregnant women. ID pups at P10 had more severe anemia, with significantly decreased hemoglobin, hematocrit, serum iron, transferrin saturation and liver iron. ID worsened the impact of alcohol upon cerebellar function at P32-34, as measured by frequency and strength of the conditioned response in eyeblink classical conditioning. Preliminary data may indicate cerebellar dysmorpholgies that are most severe in ID pups fed 3.5 g/kg/day ethanol. While results are preliminary, the structure-function relationship demonstrated thus far may indicate that gestational ID worsens the neurodevelopmental outcomes of alcohol exposure in the rat (Supported by NIH award T32 ES07015, Palmer Fund of the Waisman Center)

1693 ETHANOL INHIBITS HIPPOCAMPAL NEURON DIFFERENTIATION INDUCED BY CARBACHOL-TREATED ASTROCYTES.

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In utero exposure to ethanol has been associated with CNS alterations in the offspring. Astrocytes can modulate many neuronal functions including dendrite and axon outgrowth by secreting neuritogenic proteins. Astrocytes express muscarinic receptors whose stimulation induces activation of multiple signaling pathways, and ethanol strongly and selectively inhibits carbachol-activated phospholipase D

(PLD) signaling in these cells. We investigated whether stimulation of muscarinic receptors in astrocytes would modulate neurite outgrowth in hippocampal neurons, and whether ethanol, by interfering with muscarinic receptor signaling, would inhibit the effect of carbachol. Hippocampal neurons, co-cultured with astrocytes previously exposed to the cholinergic agonist carbachol, displayed longer axons and minor neurites, as measured by morphometric analysis of βIII-tubulin-immunolabeled neurons. This effect was mediated by the activation of M3 muscarinic receptors. Inhibition of the activity of the extracellular matrix protein fibronectin strongly reduced the effect of carbachol on axonal and minor neurite elongation, while inhibition of laminin activity reduced only the elongation of minor neurites. Co-incubation of astrocytes with ethanol (25-100 mM) and carbachol resulted in a concentration-dependent inhibition of carbachol's effects. 1-Butanol, which, like ethanol, is a competitive substrate for PLD, but not its analogue tert-butanol, which is not a PLD substrate, strongly inhibited axonal growth stimulated by carbachol-treated astrocytes, suggesting that the effect of ethanol was mediated by inhibition of PLD signaling. These findings suggest that ethanol, by inhibiting PLD signaling, affects carbachol-induced release of neurite-promoting factors by astrocytes, which ultimately lead to inhibition of neuronal differentiation. (Supported in part by AA08154 and P30ES07033).

1694 ETHANOL INHIBITS MUSCARINIC RECEPTOR-INDUCED RELEASE BY ASTROCYTES OF EXTRACELLULAR PROTEINS INVOLVED IN NEURONAL DEVELOPMENT.

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Fetal ethanol exposure can induce CNS deficiencies including alterations of neuronal and glial cells. Astroglia are particularity sensitive to the effect of ethanol. We have previously shown that in astrocytes, ethanol inhibits the mitogenic action of the cholinergic agonist carbachol, by interfering with its signaling pathway activated by muscarinic M3 receptors. We have also shown that muscarinic receptor stimulation in astrocytes induces neuronal differentiation, and that this effect is inhibited by ethanol. The goal of this project was to identify proteins involved in neuronal differentiation whose release is induced by carbachol, and to assess the effect of ethanol on carbachol-induced secretion. The global protein profile of astrocyte secretions was generated using FT-ICR MS analysis. A large fraction of the identified proteins were astrocyte extracellular proteins with reported functions in neuronal development, and included several promoters of neurite outgrowth including laminin (LM), fibronectin (FN), and plasminogen activator inhibitor-1 (PAI-1, an inhibitor of extracellular matrix protein degradation). The stimulation of astrocytes with carbachol induced upregulation of mRNA levels for all three proteins, as measured by RT-PCR. Increased extracellular protein levels of LM, FN and PAI-1 were confirmed using immunocytochemistry, Western blot and ELISA techniques. The co-incubation of ethanol with carbachol prevented carbachol-induced release of FN and PAI-1, but not LM. In conclusion, carbachol-stimulated astrocytes modulate neuronal differentiation through the release of the neuritogenic proteins LM, FN and PAI-1. These results suggest that a novel mechanism by which in utero alcohol exposure may exert its toxic effects on the developing brain is through the inhibition of astrocyte-released factors involved in neuronal differentiation. (Supp. in part by AA08154, ES07033 & ES07032)

1695 THE ROLE OF NRF2 IN DOMOIC ACID-INDUCED EFFECTS ON EARLY NEURODEVELOPMENT.

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Domoic Acid (DA), a neurotoxin found in shellfish, causes short-term memory loss, brain damage, and death in humans. In adult rodents, DA has been observed to induce neuronal death in the hippocampus and amygdala, however, little is known about the effect of DA on the developing brain. Previous studies suggest that DA hyperactivates ionotropic glutamate receptors resulting in oxidative stress. The Nrf2 gene is a key transcriptional factor for antioxidant response element(ARE)-regulated genes and the Nrf2(-/-) mouse represents a potential sensitive model to DA induced oxidative stress due to the reduced antioxidant response. We hypothesize that DA alters neuronal differentiation and proliferation during early CNS development and the deletion of the Nrf2 gene potentiates DA's effects. In this study, we characterized the effect of DA exposure on early neuronal development using two mouse embryonic neuronal cell culture systems, neuronal precursor cells (NPCs) isolated on GD11 and embryonic mixed cortical neurons isolated on GD16. We assessed for DA-induced cytotoxicity using the neutral red, and LDH functional assays and morphology examination. Furthermore, we examined the impact of DA on neuronal proliferation and differentiation by assessing protein expression of structural and synaptic markers (MAP2, alpha-synuclein, beta-tubulin), glutamate receptor subtypes (Kainate, AMPA, NMDA), and proliferative markers (PCNA). We did not observe DA-induced cytotoxicity (<100uM) after 9 days culture in our NPC system, suggesting inadequate glutamate receptor development. In the cells isolated from both wildtype and Nrf2 knockout mice on GD16, treatment with increasing doses (0- 20uM) of DA for 2, 3, and 5 days induced dose-dependent cytotoxicity. However, no significant difference between genotypes was observed. Our studies suggested the critical role of glutamate receptor of DA induced neurodevelopment and the role of Nrf2 mediated antioxidative response pathway is still needed to be further examined. Supported by: NIEHS, P50 ES012762 and NSF, OCE-0434087

1696 THE PROTECTIVE EFFECT OF L-CARNITINE ON PHENCYCLIDINE-INDUCED CORTICAL APOPTOSIS IN THE DEVELOPING RAT.

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Previous data suggest that exposure of neonatal rats to various anesthetics, including NMDA antagonists such as phencyclidine (PCP), results in profound behavioral abnormalities in adolescent rats that may be related to NMDA receptor upregulation (compensatory). We hypothesized that this upregulation makes neurons bearing this receptor more vulnerable, after PCP washout, to the excitoxic effects of glutamate, because this up-regulation allows for the accumulation of toxic levels of intracellular free calcium under normal physiological condition. L-carnitine plays an integral role in attenuating the brain injury associated with mitochondrial neurodegenerative disorders. On postnatal days 7, 9, and 11 rat pups were pretreated with either vehicle or L-carnitine (300 mg/kg) 30 minutes prior to administration of either saline or PCP (10 mg/kg). The rat pups were perfused 6 hours after the last injection for histochemical determination. PCP (10 mg/kg) resulted in a substantial enhancement of neurotoxicity as indicated by an increased number of Fluoro-Jade C-positive and caspase-3 immuno-reactive neurons, as well as grain density of silver-staining positive neurons in layers II and III of the frontal cortex. Co-incubation of L-carnitine (300 mg/kg) with PCP (10 mg/kg) reduced PCP-induced neuronal cell death. These results indicate that PCP administration causes enhanced apoptosis in rat frontal cortex. Co-administration of L-carnitine prevented this increase caused by PCP. Collectively, these data suggest that behavioral abnormalities following perinatal PCP administration result from loss of neurons in the frontal cortex.

1697 FLUORESCENT MOLECULAR IMAGING OF TRANSGENIC MICE FOR NEUROTOXICITY AND COMPOUND SCREENING.

L. Zhuo, G. Ho, S. Kumar, C. Zhang, X. Min and I. Kng. *Institute of Bioengineering and Nanotechnology, Singapore, Singapore.* Sponsor: J. O'Callaghan.

The central nervous system (CNS) is vulnerable to injury inflected by neurotoxicants, neurodegeneration, viral infection, and stroke, etc. As a universal defense mechanism, the brain responds to virtually all types of insults by astrocytic activation (hypertrophy) and/or proliferation (hyperplasia), which is termed reactive gliosis. A hallmark of the gliosis is the up-regulation of the astrocytic marker GFAP (glial fibrillary acidic protein), often preceding the microscopically observable structural alterations. Conversely when the neural insult disappears, the gliosis would also gradually return to the basal level. To achieve a real-time monitoring of gliosis in vivo, we have generated transgenic mice expressing GFP (green fluorescent protein) specifically in astrocytes under the control of GFAP promoter. In this study, we demonstrate that the neurotoxicant-induced gliosis in the neonatal brain and in the adult retina respectively can be non-invasively measured and quantified using fluorescent imaging. For a proof of concept, we further employ this imaging platform to evaluate a class of small compounds in a mouse model of Parkinsonism, and have identified candidates with a reduced neurotoxicity and an increased neuronal protection efficacy in the substantia nigra pars compacta (SNpc). Therefore the current fluorescent imaging method represents a relatively simple solution for real-time screening of neurotoxicity and efficacy of compounds with a reasonable throughput in vivo, without using a substrate or a more complicated imaging modality, such as positron emission tomography or magnetic resonance imaging.

1698 EVALUATION OF HUMAN NEURAL PROGENITOR CELLS FOR DEVELOPMENTAL NEUROTOXICITY SCREENING: TIME COURSE OF EFFECTS ON CELL PROLIFERATION AND VIABILITY.

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Current testing methods for developmental neurotoxicity (DNT) make evaluation of the effects of large numbers of chemicals impractical and prohibitively expensive. As such, we are evaluating human neural progenitor cells (NPCs) as a screen for

DNT. ReNcell CX (ReN CX) cells are an immortalized NPC line that differentiates into neurons and glia. The goal of the present study was to determine the optimal time for proliferation and cytotoxicity assays. Cell proliferation (BrdU incorporation) and viability (uptake of propidium iodide) were assessed in cells treated with a selected set of compounds (1 nM – 100 μM) for 4, 24, or 48 hrs, and each endpoint was quantified using an automated, high content imaging approach. Removal of EGF and FGF-2 from the expansion media, which initiates differentiation, decreased BrdU incorporation 12% (4 hrs), 22% (24 hrs), and 41% (48 hrs) without affecting viability. The anti-proliferatives aphidicolin and hydroxyurea inhibited BrdU incorporation most potently at 4 hrs (IC50aph = 0.21 μM; IC50HU = 23.81 μM) compared to incubation times of 24 or 48 hrs; decreases in viability were also seen at 24 (100 μM) and 48 hrs (1-100 μM). The developmental neurotoxicants methylmercury, trans-retinoic acid, and cadmium potently inhibited only proliferation at 4 hrs, but both proliferation and viability at 24 and 48 hrs, indicating that a 4-hr treatment may be most appropriate to study anti-proliferative effects in the absence of corresponding decreases in viability. Amoxicillin, acetaminophen, diphenhydramine, saccharin, sorbitol, and glyphosate (≤ 30 μM) were without effect on proliferation or viability at all treatment times. These data demonstrate the utility of ReN CX cells for screening for proliferation and viability using automated, high content imaging methods. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy).

1699 COMPARISON OF NEUROSCREEN-1 AND CEREBELLAR GRANULE CELL CULTURES FOR EVALUATING NEURITE OUTGROWTH USING THE ARRAYSCAN HIGH CONTENT ANALYSIS SYSTEM.

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A major challenge facing the Environmental Protection Agency is the development of high-throughput assays for chemical screening and toxicity prioritization. One approach uses *in vitro*, cell-based assays which recapitulate biological events observed *in vivo*. Neurite outgrowth is a critical cellular process underlying nervous system development that can be quantified using high content analysis technology. This study compared the PC-12 neuronal cell line (NS-1), and primary neuronal cultures of cerebellar granular cells (CGC) as potential models for assessing chemical effects on neurite outgrowth. High content analysis of neurite outgrowth was performed using the Cellomics ArrayScan V^{Ti} automated epifluorescent imaging system to acquire and analyze images of β-tubulin immunostained cells in 96-well plates. Incubation in NS-1 or CGC in NGF or serum respectively, induced neurite outgrowth within 24 hours and continued to elongate over several days *in vitro*. The MEK inhibitor U0126 (1 – 30 uM) and the PKC inhibitor Bis-1 (0.3 – 10 uM), known positive controls, inhibited neurite outgrowth in both NS-1 and CGC. Neurite outgrowth was further evaluated using eight chemicals that are known developmental neurotoxicants *in vivo*. Cells were exposed for either 4 (NS-1) or 2 (CGC) days to 1 nM - 100 uM. Exposure to methylmercury or cadmium reduced neurite outgrowth in both models. Inhibition of neurite outgrowth by trans-retinoic acid and dexamethasone was more pronounced in NS-1 cells compared to CGC. In contrast, lead and amphetamine inhibited neurite outgrowth only in CGC. These results suggest differences in sensitivities of *in vitro* models to chemical effects on neurite outgrowth. Future research will evaluate additional developmental processes, including proliferation and synaptogenesis, for screening using high content analysis. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy).

1700 EFFECTS OF PRENATAL EXPOSURE TO 1-BROMOPROPANE ON NEURONAL EXCITABILITY IN THE HIPPOCAMPUS OF OFFSPRING.

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1-Bromopropane (1-BP), a substitute for specific chlorofluorocarbons, is mainly used for degreasing agents and spray adhesives. 1-BP exhibits central neurotoxicity in adult humans, and our recent studies have shown that 1-BP exposure alters the neurotransmitter receptor function, intracellular signaling and feedback inhibition in *in vitro* and *in vivo* model system (Ueno *et al.*, Yoshida *et al.*, Fueta *et al.*, Neurotoxicology vol 28, 2007). However, effects of 1-BP exposure during the pregnant period on the neuronal development remains unclear. Thus, we aimed to clarify whether prenatal exposure to 1-BP affects development of neuronal excitability in the offspring. 1-BP was inhalationally exposed to 48 pregnant Wistar rats from day 1 to 20 (6 h/day) with the concentration of 0, 400, or 700 ppm. On the days of PND 13, 14 and 15, and the adult hood (6-8 w) of the male offspring, hippocampal slices were prepared. Field potentials were recorded from the cell layer and stratum radiatum of the CA1 region. Stimulation/response (S/R) curves of field excitatory postsynaptic potential (fEPSP) and of population spike (PS), representing basic excitability of neurons, were examined in pregnant rats and their offspring. In the 700-ppm group, 1-BP caused hyperexcitability in the pregnant rats and inhibition of body weight increase during development in the offspring. S/R curves of fEPSP and/or PS were enhanced in the exposure groups at PND 14 and 15. On the contrary, the S/R curves in adulthood were suppressed in the 700-ppm group. Our results provide the evidence that prenatal exposure to 1-BP/metabolites affects the central nervous system of pregnant rats and their offspring, suggesting disruption of basic neuronal excitability of the hippocampal formation.

1701 RETINOIC ACID AFFECTS CHOLESTEROL HOMEOSTASIS IN ASTROCYTES: A NEW MECHANISM OF TERATOGENESIS?

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Excess of retinoic acid causes teratogenesis both in humans and animal models via activation of the nuclear retinoic acid receptors RXR and RAR. Cholesterol is essential during fetal development and, in particular, brain development. The effects of excess retinoic acid on brain development present similarities with ethanol-induced teratogenesis and are consistent with abnormalities caused by lack of cholesterol. We have recently shown that ethanol, by upregulating ABC cholesterol transporters in astrocytes, increases cholesterol efflux leading to cholesterol depletion in these cells and have hypothesized that this could be a mechanism by which ethanol induces some of its teratogenic effects. We hypothesized that, similarly to ethanol, retinoic acid may upregulate cholesterol transporters and cholesterol efflux in fetal rat astrocytes leading to cholesterol depletion. ABCA1 were indeed upregulated by three retinoic acid isomers, All Trans Retinoic Acid (ATRA), 9-cis retinoic acid (9-cisRA), and 13-cis retinoid acid (13-cisRA or isotretinoin), that activate RAR/RXR heterodimers, but not by the retinoic acid precursor retinol (vitamin A) nor the selective RAR and RXR ligands TTNPB and methoprene, suggesting that the activation of RXR/RAR heterodimers is necessary for this effect. ATRA, 9-cisRA and 13-cisRA also increased the levels of another cholesterol transporter, ABCG1. Consistent with the notion that ABCA1 and ABCG1 are involved in cholesterol efflux, we also found that 13-cisRA and 9-cisRA induced cholesterol efflux in astrocytes with a maximal effect at 10 μM and 1 μM respectively. Finally, we found that 13-cisRA and 9-cisRA reduced the intracellular levels of cholesterol. Taken together these findings suggest that a common mechanism may be responsible for ethanol and excess retinoic acid teratogenesis involving the alteration in cholesterol homeostasis in astrocytes leading to cholesterol depletion (Supported in part by AA015443, AA08154, and P30ES07033).

1702 PATHOLOGICAL ASSESSMENT OF THE NERVOUS SYSTEM OF RAT OFFSPRING EXPOSED TO ACRYLAMIDE DURING THE GESTATION AND LACTATION PERIODS – A PRELIMINARY STUDY.

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To evaluate the developmental exposure effect of acrylamide (ACR) on the nervous system, pregnant Sprague-Dawley rats were given ACR at 0, 50, 100 or 200 ppm in the drinking water from gestational day 10 to postnatal day (PND) 21 and histopathological assessment of offspring was performed at weaning (PND 21) and postnatal week (PNW) 11. Neurotoxicity was quantitatively assessed with reference to nerve fiber density, percentages of degenerated and small caliber axons in the sciatic nerves, and numbers of aberrant dot-like structures immunoreactive for synaptophysin in the cerebellar molecular layer. In addition, a group of rat neonates that received ACR at 50 mg/kg by intraperitoneal injections 3 times a week from PND 2 to 21 was subjected to analysis for comparison with maternal exposure groups. Dams showed dose-related lowered body weights and neurotoxicity, both of them being apparent during the lactation period after delivery. Offspring exposed to ACR maternally also showed lowered body weight from PND 2 through weaning from 50 ppm in males and from 100 ppm in females, the lowered body weight being remained until PNW 11. However, no histopathological changes as well as gait abnormality suggestive of neurotoxicity were observed in these ACR-exposed offspring at both PND 21 and PNW 11. In contrast, offspring given ACR intraperitoneally revealed gait abnormality and peripheral nerve axonal degeneration. Slight reduction of neonatal body weight examined shortly after birth was considered to be due to developmental effect of ACR administered during gestation as reported by others. While ACR injected intraperitoneally caused neurotoxicity, no

signs of neurotoxicity were found in offspring exposed to ACR maternally. Therefore, poor lactational ACR-exposure due to maternal toxicity might account for the lack of ACR-induced offspring toxicity other than retarded body growth.

1703 DEVELOPMENTAL EXPOSURE TO PERCHLORATE ALTERS SYNAPTIC TRANSMISSION IN HIPPOCAMPUS OF THE ADULT RAT.

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The Food Quality Protection Act and Safe Drinking Water Act mandate the EPA to identify potential health risks associated with chemicals that act on the endocrine system. Perchlorate, a contaminant found in food and water supplies throughout the USA, blocks iodine uptake into the thyroid gland and reduces circulating levels of thyroid hormone (TH). The present study was designed to evaluate the potential neurological effects of TH reductions induced by developmental exposure to perchlorate. Pregnant Long-Evans dams were exposed to 0, 30, 300 and 1000ppm perchlorate in the drinking water from gestational day 6 until pups were weaned on postnatal day 30. T4, T3 and TSH were determined in male and female offspring at four ages (PN4, PN14, PN21, PN80-90). Adult male offspring were evaluated on a series of behavioral tasks and electrophysiological measures of synaptic function in the hippocampus. T3 and T4 were reduced in pups on PN21 at the highest dose level. T4 in dams was reduced 12%, 24% and 56% in the 30, 300 and 1000ppm dose groups, respectively. Reductions in T4 were associated with an increase in TSH limited to the high dose group and no changes were seen in serum T3. No effects of perchlorate were detected on behavioral measures of motor activity, spatial learning, or fear conditioning. However, in field potentials recorded from the dentate gyrus, reductions in excitatory postsynaptic potentials (EPSP) and population spike (PS) measures were detected. Significant reductions in baseline synaptic transmission of the PS were observed at all dose levels. Reductions in paired inhibitory synaptic transmission were also evident at 300 and 1000ppm dose levels. These findings indicate an irreversible impairment in synaptic transmission of the hippocampus in response to developmental exposure to perchlorate. Deficits were dose-dependent and detectable with relatively minor perturbations of the thyroid axis. (This is an abstract for a proposed presentation and does not necessarily reflect EPA policy)

1704 THE ROLE OF N-METHYL-D-ASPARTATE RECEPTORS IN POLYCHLORINATED BIPHENYL-MEDIATED NEUROTOXICITY.

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Polychlorinated biphenyls (PCBs) are widespread persistent environmental pollutants. Chronic human and animal exposure to PCBs results in various harmful effects including neurotoxicity. This study investigates the effects of the PCB mixture Aroclor 1254 and the PCB congeners 3,3',4,4',5-pentachlorobiphenyl (PCB 126, coplanar, non-ortho) and 2,2',4,4',5-pentachlorobiphenyl (PCB 99, non-coplanar) on the expression of N-methyl-D-aspartate receptors (NMDA-R) and the subsequent induction of cell death using a SHS5-SY human neuroblastoma cell line. NMDA-R was measured using the radiolabeled phencyclidine receptor ligand [3H] MK-801 and cell death was quantified using fluorogenic substrates specific for caspase-3 (DEVD-AFC) and lactate dehydrogenase (LDH) release. After 4 and 24 hours incubation, a positive dose response relationship was observed in all PCB compounds (10 to 50 μM) and the non coplanar PCBs compounds were found to be significantly more toxic. The PCB-mediated cell death was inhibited with 15μM NMDA-R antagonists: 1-amino-3,5-dimethyladamantane hydrochloride (memantine) and (+)-5-methyl-10,11-dihydro-5H-debenzocyclhepten-5,10-imine maleate ((+)-MK-801), thus demonstrating the importance of NMDA-R in PCB neurotoxicity. Intracellular calcium chelator BAPTA-AM (1μM) partially attenuated the neurotoxicity effect of PCB. In summary, these results suggest that a receptor mediated mechanism for PCB neurotoxicity.

1705 POLYCHLORINATED BIPHENYLS (PCBs) INFLUENCE DENDRITIC GROWTH IN CULTURED HIPPOCAMPAL NEURONS VIA RYANODINE RECEPTOR (RYR)-DEPENDENT ACTIVATION OF CAM KINASE I.

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Developmental exposure to PCBs is linked to cognitive deficits in humans and experimental animals, but the mechanism(s) underlying the developmental neurotoxicity of these compounds remain speculative. Our previous in vivo studies of rats exposed developmentally to PCBs demonstrated that PCBs disrupt spatial learning and memory coincident with altered dendritic growth and ryanodine receptor (RyR) expression. Here we show that PCB effects on dendritic growth and RyRs are causally related, and identify downstream signaling pathways. We quantified dendritic growth in cultured hippocampal neurons transfected with a GFP-tagged MAP2 construct following a 48 h exposure to either PCB95 (2,2',3,5',6-pentachlorobiphenyl), a non-coplanar PCB congener that is a potent activator of the RyR, or PCB66 (2,3',4,4'-tetrachlorobiphenyl), a coplanar PCB congener with no activity at the RyR. PCB95, but not PCB66, significantly enhanced dendritic growth. The dendrite promoting activity of PCB95 was evident at concentrations as low as 2 pM and was completely blocked by FLA365, a RyR channel blocker. PCB95 similarly enhanced dendritic growth in hippocampal slice cultures. Activity-dependent dendritic growth in hippocampal neurons requires sequential activation of CaM-dependent kinase kinase (CamKK), CamKI and MEK/ERK to enhance CREB-mediated transcription of Wnt. Genetic and pharmacological blockade of these signaling molecules inhibits PCB95-induced dendritic growth. These data identify a novel mechanism of PCB developmental neurotoxicity in which PCB activation of RyR modulates dendritic plasticity, and suggest that non-coplanar PCBs may be useful probes for studying the role of the RyR in activity-dependent dendritic growth. This work supported by NIH grants ES014901 (PJL and INP), ES11269 (INP) and GM041292 (GAW).

1706 INDUCTION OF NEUROTOXICITY IN FETAL BRAIN AFTER PRENATAL EXPOSURE TO VALPROATE.

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We have previously reported that inhibition of neuronal differentiation was observed in the fetal rat forebrain on embryonic day (ED) 14 (plug day= ED0) when 800 mg/kg of valproic acid (VPA) was administered orally to the dam on ED9 (VPA9) or 11 (VPA11), which has been reported to produce an animal model of autism. However, it was not clear whether the effect was due to VPA–induced neurotoxicity (direct effect) or just developmental retardation (secondary effect).
In this study, the timing of the observation was set at two days later (ED16), and the effects of VPA exposure on early development of the central nervous system were examined. Both VPA9- and VPA11-treated groups showed displasia of the cortical plate. Neurites detected by TuJ1 immunohistochemistry were considerably fewer in the subplate layer in the VPA11-treated group. These changes in VPA11 treatment were more severe than those in VPA9 treatment. In addition, the pons in VPA11-treated fetuses showed fewer migrating cells and was not fused, which seemed to reflect a younger stage of development.
In conclusion, VPA both at ED 9 and 11 treatment affects the development of the cortical plate in the cerebral cortex. VPA11 treatment affects the development of the pons. In the model for autism induced by prenatal VPA exposure, fetal brain shortly after exposure showed heterogeneous VPA-induced neurotoxicity depending on the developmental stage and/or brain areas.

1707 IN UTERO AND LACTATIONAL EXPOSURE TO A LOW DOSE OF TCDD OR TBDD PERTURBS PAIRED ASSOCIATIVE LEARNING OF MALE RAT OFFSPRING.

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In utero and lactational exposure to dioxins is known to result in behavioral alterations in the offspring of laboratory animals. However, it has not been well-characterized how dioxins affect advanced brain functions, such as learning and memory. Recently, a novel sophisticated learning test for rats named the "Flavor Map test" was developed (Tse, D. et al., Science, 316:76-82,2007). This test makes it possible to evaluate paired-associative learning performance in the rat. Here, we studied effects of *in utero* and lactational exposure to a low dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 2,3,7,8-tetrabromodibenzo-*p*-dioxin (TBDD) on paired-associative learning in male rat offspring. Pregnant Long-Evans rats were given orally either TCDD or TBDD at a dose of 0, 200 or 800 ng/kg on gestation day 15. It was found that *in utero* and lactational exposure to TCDD at 800 ng/kg resulted in a 10-15% decrease of body weight in adulthood (postnatal day 91 and later). A similar trend was also observed in rat offspring born to dams given TBDD at 800 ng/kg. Before starting the Flavor Map test, rat offspring born to dams exposed to TCDD or TBDD at 200 ng/kg exhibited anxiety-like behavior at the time of habituation to the "event arena" of the test apparatus. In the Flavor Map test, control animals had a normal success rate in learning paired-association of the reward place and a particular flavor within 5 weeks. In contrast, rat offspring born to dams exposed to 200 ng TCDD/kg had a lower success rate than control

offspring for the acquisition of paired-associative learning. Thus, the present study demonstrate that *in utero* and lactational exposure to a very low dose of TCDD or TBDD impaired advanced learning ability and caused anxiety in male rat offspring in adulthood. [This Study was supported, in part, by the Ministry of the Environment, Japan.]

1708 HIGHLY BROMINATED DIPHENYL ETHERS, PBDE 209, 206 AND 203 AFFECT DEVELOPMENTAL MARKER PROTEINS IN THE NEONATAL MOUSE BRAIN.

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Polybrominated diphenyl ethers (PBDEs) are used in large quantities as flame-retardants. In Europe and USA only the fully brominated PBDE 209, 2,2',3,3',4,4',5,5',6,6'-decaBDE, is still in use. Recent studies report of increasing PBDE 209 levels in biota and humans. PBDE 209 is present in human mother's milk and higher levels have been found in toddlers compared to adults. In a recent study we have seen that neonatal exposure to PBDE 209, as well as nona- and octaBDEs, can cause permanent aberrations in spontaneous behaviour and habituation, effects that also worsen with age, in mice and rats. Our data suggest that the developmental neurobehavioural effects of PBDE 209 were induced when metabolites of PBDE 209 were present during a critical phase of neonatal brain development. In view of our earlier results and the increasing exposure to PBDE 209 the present study was undertaken to investigate possible neurochemical changes in the neonatal brain after PBDE 209 exposure as well as exposure to two of the possible metabolites of PBDE 209, namely PBDE 203 and PBDE 206. Neonatal mice, exposed to PBDE 209 (21 μmol/kg bw.) on postnatal day (PND) 3, were sacrificed 7 days post-treatment and neurochemical changes were studied in different brain regions. Another group of mice were exposed to PBDE 203 or 206 (21 μmol/kg bw.) on PND 10 and sacrificed 24 h post-treatment and neurochemical changes were studied in different brain regions. Animals exposed to PBDE 209 on PND 3 showed significantly changed levels of CaMKII and GAP-43 in hippocampus and cortex 7 days post-treatment (around PND 10). Animals exposed to PBDE 203 on PND 10 showed significantly changed levels of CaMKII in hippocampus 24 hours post-treatment. This supports earlier findings indicating metabolism of PBDE 209 to neurotoxic metabolites. In conclusion, PBDE 209 can affect the postnatal neurodevelopment in mouse and one possible mechanism behind the neurotoxicity of PBDE 209 is the debromiation of the parent compound to other highly brominated diphenyl ethers.

1709 EXPRESSION AND FUNCTIONAL CHARACTERIZATION OF THE ORGANIC CATION TRANSPORTER-3 IN MOUSE ASTROCYTES.

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The contribution of environmental toxicants to neurodegenerative diseases has been a focus of biomedical research for many years, but much remains to be learned about how these chemicals are delivered to their site of action, how their concentrations are regulated, and how they exert their neurotoxic effects in the brain. We hypothesize that the organic cation transporter-3 (OCT3), also known as the extraneuronal monoamine transporter, contributes to the selective damage in the nigrostriatal region as seen in the MPTP mouse model of Parkinson's disease, where this prototoxicant is converted to the toxic metabolite MPP+ inside of astrocytes. However, the expression of this transporter in the mouse brain and its transport properties in mouse astrocytes have not been characterized. In adult C57BL mice (~ 10 weeks old), immunohistochemistry followed by confocal analyses showed that OCT3 was selectively expressed in astrocytes in the nigrostriatal region but not in the astrocytes that reside in the locus coeruleus, raphe nuclei, cerebellum, hippocampus, and cortex. A similar pattern of expression was also detected in postmortem human brain sections, supporting the relevance of this transport to humans. Functional transport studies in cultured primary astrocytes and cells stably overexpressing OCT3 confirmed the ability of OCT3 to reverse transport MPP+, thus supporting its ability to modulate cell death in the MPTP toxicity. Furthermore, in vivo microdialysis data obtained from free moving mice injected with MPTP showed extracellular striatal levels of MPP+ were significantly lower in mice with OCT3 deficient than those in the wild type littermates. Together these data support our previous report that MPTP induced less damage to the nigrostriatal dopaminergic structures in the OCT3-knockout mice. Using transgenic mice overexpressing EGFP driven by the GFAP promoter, we are further characterizing the expression of OCT3 at different developmental stages.

1710 PERFLUOROOCTANESULFONATE DEVELOPMENTAL NEUROTOXICITY STUDY IN RATS.

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Perfluorooctanesulfonate (PFOS), a persistent and bioaccumulative compound, is widely distributed in humans and wildlife. The objective of this study was to evaluate the functional and morphological changes to the nervous system in rat pups with gestational and lactational exposures to PFOS per current test guidelines (EPA OPPTS 870.6300 and OECD 426).
Female SD rats were given daily oral doses with either vehicle control or PFOS at 0.1, 0.3, and 1.0 mg/kg-d from gestation day (GD) 0 through lactation day (LD) 20. Offspring were observed through postnantal day (PND) 72 for growth, behavioral evaluations, and brain morphology. Serum PFOS concentrations were measured using LC/MS-MS and a subset of liver samples from GD20 and PND 21 were evaluated for PPARα-related mRNA transcript expression by quantitative RT-PCR. The NOAEL for maternal toxicity was considered to be 0.3 mg/kg-d due to slightly lower mean body-weight gain and reduced food consumption in the 1.0 mg/kg-d group primarily during LD 1-4. There was no absolute evidence of PFOS-related effect on maternal thyroid status or effects on postnatal growth, survival or neonatal thyroid status. The NOAEL for F_1 developmental neurotoxicity was considered to be 0.3 mg/kg-d based on increased motor activity and reduced habituation to the test environment noted in the 1.0 mg/kg-d group F_1 males on PND 17.
At GD 20, mean serum PFOS concentrations were approximately 6 and 26 μg/mL for 0.3 and 1.0 mg/kg-d F_0 females, respectively. In fetus, mean serum PFOS concentrations were about 10 and 31 μg/mL for 0.3 and 1.0 mg/kg-d groups, respectively. At LD 21, mean serum PFOS concentrations were 9 and 30 μg/mL for 0.3 and 1.0 mg/kg-d F_0 females, respectively. At PND 21, for both male and female pups, mean serum PFOS concentration were approximately 5 and 18 μg/mL for 0.3 mg/kg-d and 1.0 mg/kg-d groups, respectively. Liver PPARα-associated mRNA transcripts were elevated in male pups on PND 21.

1711 BISPHENOL A IS RELEASED FROM POLYCARBONATE DRINKING BOTTLES AND MIMICS THE NEUROTOXIC ACTIONS OF ESTROGEN IN DEVELOPING CEREBELLAR NEURONS.

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The impact of endocrine disrupting chemical (EDC) exposure on human health is receiving increasingly focused attention. The prototypical EDC bisphenol A (BPA) is an estrogenic high-production chemical used primarily as a monomer for production of polycarbonate and epoxy resins. It is now well established that there is ubiquitous human exposure to BPA. In the general population exposure to BPA occurs by consumption of contaminated foods that have contacted epoxy resins or polycarbonate plastics. To test the hypothesis that bioactive BPA was released from polycarbonate bottles used for consumption of water and other beverages, we evaluated whether BPA migrated into water stored in new or used high-quality polycarbonate bottles used by consumers. Using a sensitive and quantitative competitive enzyme-linked immunosorbent assay, BPA was found to migrate from polycarbonate water bottles at rates ranging from 0.20 to 0.79 ng per hour. At room temperature the migration of BPA was independent of whether or not the bottle had been previously used. Exposure to boiling water (100C) increased the rate of BPA migration by up to 55-fold. The estrogenic bioactivity of the BPA-like immunoreactivity released into the water samples was confirmed using an in vitro assay of rapid estrogen-signaling and neurotoxicity in developing cerebellar neurons. While levels of human BPA exposure from the BPA drinking bottles alone are expected to be low, these levels should be considered a contributing component of the total "EDC-burden" to which individuals can be exposed.

1712 HEARING IMPAIRMENT FOLLOWING DEVELOPMENTAL EXPOSURE TO PCBs IN A RAT MODEL.

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A confluence of evidence has indicated that developmental exposure to PCBs can result in hearing impairments. These sensory deficits may contribute to the negative effects of PCBs on cognitive function observed in humans. Research in our laboratory has shown that developmental exposure to PCBs results in cochlear deficits in rats, determined by measurement of distortion product otoacoustic emissions (DPOAEs). Maternal exposure to PCBs at either 3 or 6 mg/kg/d (for 77 days up to weaning) resulted in DPOAE deficits in adult offspring. DPOAE amplitudes were

decreased and thresholds were elevated at frequencies ranging from 2 kHz to 12 kHz. These rats also showed auditory brainstem response (ABR) threshold elevations at frequencies ranging from 1.5 kHz to 48 kHz. These results have now been replicated in a subsequent study. Experiments now in progress are focused on understanding the mechanism of this PCB-induced hearing loss, and on assessing higher-level auditory processing in PCB exposed rats. PCB exposure is thought to disrupt cochlear function by thyroid hormone depletion, but altered calcium signaling via ryanodine receptor activation may also play a role. The relative contribution of these two mechanisms will be determined by assessing cochlear function in rats exposed developmentally to either PCB 52 or PCB 77. PCB 52 is an activator of the ryanodine receptor, but does not affect thyroid hormone levels; while PCB 77 does not activate the ryanodine receptor, but does cause thyroid hormone depletion. Parallel studies will focus on investigating the effects of PCBs on neural distortion products recorded via the frequency following response (FFR), an evoked potential that reflects sustained neural activity phase-locked to the stimulus waveform. FFRs have been used to assess temporal processing in humans, and an animal model may provide insight relevant to the temporal processing of sound that characterizes human speech perception. The neural distortion products could complement DPOAE and ABR results, providing new information about the impact of PCB exposure on other aspects of auditory function.

1713 RDX PILOT FOR DEVELOPMENTAL NEUROTOXICITY TEST IN RATS.

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Hexahydro-1,3,5-trinitro-1,3,5-triazine (Royal Demolition Explosive or RDX) is an explosive nitroamine widely used in military and industrial applications. Contamination with RDX has been identified at areas of explosive manufacturing, processing, storage, and usage. Thus, the potential exists for exposure to humans. There are limited data on the neurotoxic and developmental effects of RDX. To determine a neurotoxic developmental effect the compound must first be transferred from the mother to the offspring during gestation. This study was designed to determine if RDX would be passed to the offspring during gestation as well as during lactation. Female Sprague Dawley rats were dosed with RDX, 6 mg/kg on gestational day (GD) 6 through postnatal day (PND) 10. At PND 0 3, 5, and 10 dams and pups were tested for RDX in the milk and brain, respectively. The results indicated that there was a significant amount of RDX present in the brain of the pups following parturition. RDX was also found in significant amounts in the milk during lactation. Given these data further studies evaluating the neurotoxic and developmental effects of RDX should be conducted. The views expressed in this paper are those of the authors and do not necessarily reflect the views and policies of the U.S. Army.

1714 EFFECTS OF 7-NITROINDAZOLE AND MELATONIN ON KETAMINE-INDUCED NEUROTOXICITY IN POSTNATAL DAY 3 (PND-3) MONKEY FRONTAL CORTICAL CULTURES.

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Ketamine, a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist, is used as a general anesthetic in infants. Recent data suggest that anesthetic drugs may cause neurodegeneration during development. The purpose of this study was to determine the dose and temporal response of ketamine using PND-3 monkey forebrain cultures and to determine if co-administration of 7-nitroindazole, a neuronal nitric oxide synthase (nNOS) inhibitor or melatonin, a pineal hormone with antioxidant properties, could protect against or attenuate ketamine-induced cell death. Neural cells collected from the frontal cortex of PND-3 monkeys were incubated for 24 hrs with 1, 10 or 20 μM ketamine alone, and with ketamine (10 μM) plus 1, 5, 10 and 20 μM 7-nitroindazole; or 100, 200, 500 or 1000 μM melatonin. Ketamine caused an increase in DNA fragmentation, increased the release of lactate dehydrogenase (LDH), elevated immunoreactivity to nitrotyrosine and reduced mitochondrial metabolism. Ketamine-induced neurotoxic effects were effectively blocked by co-administration of 7-nitroindazole (10 and 20 μM) or melatonin (500 and 1000 μM). No significant toxic effects were observed in 7-nitroindazole- or melatonin-alone treated cultures. These data indicate a role for nitric oxide in the enhanced degeneration induced by ketamine in vitro, and suggest that blocking nNOS may help reduce the risk of using ketamine in pediatric anesthesia. These data also suggest a potential protective effect of melatonin in ketamine-induced neurotoxicity. (Supported by Interagency Agreement #224-93-0001 between the NCTR/FDA and the NIEHS/NTP)

1715 NEUROTOXICITY OF SOME NEW ANTICONVULSANT N, N'-SUBSTITUTED SPIROHYDANTOINS.

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Previously we reported the neurotxicity of an initial series of N,N'-disubstituted-spiro-phthalidyl hydantoins that were prepared by reaction of the corresponding N,N'-disubstituted ureas with ninhydrin, followed by treatment with aqueous sodium periodate oxidation. The spirohydantoins exhibited anticonvusant activitites in both the maximal electro shock (MES) and pentylene tetrazole (PTZ) tests. While N-aryl substituted hydantoins showed appreciable anticonvulsant activities in PTZ assay and less in the MES test, they also exhibited some toxicity in the righting reflex test. In the rotorod test, compounds bearing small alkyl (methyl, ethyl and propyl) N-substituents were less neurotoxic than N-aryl substituted ones. In order to minimize the toxicity a new series of spirohydantoins bearing electron-withdrawing (WD) and donating (ED) substituted aryl groups were prepared and their anticonvulsant activities and their neurotoxicity were tested. Compounds with WD groups exhibited the most anticonvulsant activity in the PTZ test and little or none in the MES assay. Most compounds exhibited neurotoxicity (rotorod) attributed to their increased lipohilicity and permability of BBB. This phenomenon was observed to a lesser extent by those bearing ED vs WD groups.

1716 TRANSFORMATION OF DEVELOPMENTAL NEUROTOXICITY DATA INTO STRUCTURE-SEARCHABLE TOXML DATABASE.

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Assessing the potential neurotoxicity of new chemicals is hampered by lack of available data. A working group was formed to construct the first publicly available, structure-searchable neurotoxicity database to increase understanding of the relationship between structure and neurotoxic activity, and to serve the neurotoxicity community across sectors of industry, government and academia. The goals are to: 1) enable data mining of biological and chemical information in relation to neurotoxicity; 2) provide a foundation for making chemical inferences and safety assessments; 3) develop a knowledge-base to support development of in vitro and in silico methods. The relational ToxML data model was adopted to enable query strategies for translating vast amounts of data into knowledge, such as neurotoxicity rules or alerts. Initially concentrating on developmental neurotoxicity (DNT) studies, a set of database fields and controlled vocabulary terms for neurotoxicity are proposed, largely based on the ILSI RF Prototype developmental toxicity database: http://rsi.ilsi.org/Projects/devtoxsar.htm. In the ToxML database, a compound is represented hierarchically by studies, tests, dosage regimens and time lines, with maternal and offspring effects aggregated for each dose group. Offspring toxicity includes cage-side observations, functional tests, and neurohistopathology. As a pilot study, a small number of publications were entered in the ToxML Editor and reviewed by the group. The resulting ToxML data model and controlled vocabulary are presented. The pilot DNT database is open and freely available (http://www.leadscope.com/toxml_editor) and provides a powerful tool for exploring biological and chemical elements relevant to the neurotoxic potential of chemicals. (This abstract does not represent USEPA or US FDA policy)

1717 BNIP3 INDUCES MITOCHONDRIAL DYSFUNCTION AND CELL DEATH THROUGH MODULATING ENDOPLASMIC RETICULAR AND MITOCHONDRIAL CA2+ STORES.

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Cyanide is a potent neurotoxin that induces cell death in selective brain areas, with dopaminergic mesencephalic cells being the most sensitive. We have showed that cyanide upregulates BNIP3 (a BH-3 only member of the Bcl-2 family) to produce apoptosis in immortalized dopaminergic cells (Mes 23.5 cells). In this study, cyanide induced endogenous BNIP3 expression in both mitochondria (MT) and endoplasmic reticulum (ER). To restrict expression of BNIP3 to MT or ER, mutants with subcellular targeting sequences were constructed by substitution of the BNIP3 transmembrane domain (TM) with that of Acta (BNIP3-Acta) for the MT and cytochrome B5 (BNIP3-Cb5) for the ER. Overexpression of BNIP3 wild-type, BNIP3-Acta or BNIP3-Cb5 induced apoptosis in a time-dependent manner, whereas the TM truncated mutant of BNIP3 failed to produce cell damage. BNIP3 wild-type and BNIP3-Cb5, but not BNIP3-Acta, led to progressive depletion of the ER Ca2+ pool and subsequent Ca2+ accumulation in MT. Pretreatment with

Z-VAD-fmk, a pan-caspase inhibitor, did not alter the redistribution of Ca2+ to MT. However, the increase in mitochondrial Ca2+ was suppressed by a specific inhibitor of mitochondrial Ca2+ uniporter, Ru-360. Overexpression of BNIP3 wild-type, BNIP3-Acta or BNIP3-Cb5 led to MT dysfunction (reduced membrane potential) without activation of caspase pathway. Cyclosporin A (CsA), an inhibitor of MT permeability transition (MPT), blocked BNIP3 wild-type, BNIP3-Acta and BNIP3-Cb5-induced collapse of $\Delta\Psi m$ and reduced the apoptosis. Ru-360 also reduced BNIP3 wild-type and BNIP3-Cb5 induced MPT and cell death, but had no effect on BNIP3-Acta induced cell damage. It was concluded that cyanide upregulated BNIP3 expression in both the ER and MT. Overexpression of BNIP3 altered Ca2+ levels in both the ER and MT, which in turn resulted in MT-mediated, caspase-independent apoptotic cell death. (This work was supported by National Institutes of Health grant ES 04140)

1718 PROTEOMIC ANALYSIS OF PBDE-99 EXPOSED *IN VIVO* CORTEX AND *IN VITRO* CORTICAL CELLS.

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Polybrominated diphenyl ethers (PBDEs) are chemicals widely used as flame retardants. PBDE-99 is one of the most commonly found PBDE congeners in human and environmental samples. Several research groups have independently shown that neonatal exposure has the potential to adversely affect neurodevelopment. We have earlier shown that PBDE-99 changes the expression pattern of proteins involved in neurite sprouting in striatum and energy metabolism in hippocampus. Here we wanted to more closely characterize the effects of PBDE-99 on cortical development by analyzing the PBDE-99 induced effects on protein expression in neonatal mouse cortex using two-dimensional difference in gel electrophoresis (2D-DIGE). In addition, we have analyzed the effects on cortical cells in vitro, using a primary cortex culture from embryonal rat brains. This parallel approach allows for comparative in vivo-in vitro studies of cortical effects. 24 hours after PBDE-99 exposure, proteins were extracted from PND10 mouse cortices, and pre-fractionated into cytosol and membrane fractions prior to 2D-DIGE analysis. After analysis, proteins of interest were picked, digested and identified using a linear ion trap mass spectrometer. In parallel, a primary cortex culture was established from embryonal rats. The cells were cultured for 24 hours, exposed to 3, 10 or 30 μM PBDE-99 for 24 hours. After 2D-DIGE analysis, the proteins were identified as in the in vivo experiment. We show that the expression of at least 127 identified proteins were changed in the cortex, many of which 35% are associated with the cytoskeleton. The in vitro data show that many proteins show an altered expression already at the lowest dose, and a dose dependent expression pattern. Our studies will not only add valuable data on the mechanisms of action of PBDE-99 in the developing mouse cortex, but also add to the knowledge on in vivo-in vitro comparisons.

1719 METHAMPHETAMINE RESPONSE IS DEPENDENT ON GENOTYPE, AGE, AND INITIAL DOSE.

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A large percentage of drug addictions begin in adolescence. As such, we set out to determine whether neurotoxic methamphetamine (MA) exposure during adolescence could produce genotype-dependent changes in adult behavior utilizing open-field activity. C57BL/6, DBA/2 and 129S6SvEv/Tac mouse strains were selected based on different behavioral reactions to MA during preliminary studies. A neurotoxic binge dose of MA was administered in adolescence (PND 40) followed by behavioral testing with a non-toxic activating dose at adulthood (PND 80) with the hypothesis that MA pretreatment would increase locomotor activity when compared to saline controls. Two additional studies were conducted to determine whether resulting behaviors were due to age, toxicity, or an interaction of the two. These studies separately investigated behavioral responses to non-toxic dosing during adolescence and adulthood, and neurotoxic binge dosing during adulthood. The neurotoxic 5 mg/kg MA doses were given four times a day in 2h intervals across two days, while the administration of the non-toxic 1 mg/kg MA doses occurred once a day over two days. Animals were injected with a 1 mg/kg MA challenge dose and tested for 1h in an open-field activity box forty days after their first injection. Control animals received saline in the same intervals as the MA treated animals. C57BL/6 animals display behavioral activation due to an adolescent MA pretreatment effect for both the toxic and non-toxic doses ($p<0.05$) with no effect seen in the adult neurotoxic study. DBA/2 adolescent mice exhibited no significant MA pretreatment activation for either dosing schedule. However, an effect was found between two testing groups in the adult neurotoxic study ($p<0.05$). 129S6SvEv/Tac animals demonstrated significant MA pretreatment activation in the adult toxicity paradigm ($p<0.01$) but no effect in the adolescent studies. The differential induction of behavioral activation 40 days following MA pretreatment indicates complex interactions between the genotype, age, and dose of the MA pretreatment.

1720 D-β-HYDROXYBUTYRATE, A PRODUCT OF THE KETOGENIC DIET, IS NEUROPROTECTIVE IN ANIMAL MODELS OF HUNTINGTON'S DISEASE BY TARGETING BOTH MITOCHONDRIAL AND EPIGENETIC MECHANISMS.

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disease caused genetically by the expansion of CAG repeats which could be exacerbated by environmental factors. Abnormal mitochondrial function and epigenetic modifications are two of the proposed pathogenic mechanisms. Currently, there is no effective treatment for HD. The ketogenic diet, a high fat/low carbohydrate diet, has been used for the treatment of epilepsy and has demonstrated potential for the treatment of some neurodegenerative diseases. The mechanism of neuroprotection conferred by a ketogenic diet has not been well defined, however, elevated levels of ketone bodies has been suggested. D-β-hydroxybutyrate (DβHB) is the major ketone body that yields bioenergetic effects. Consistent with this role, in the 3-nitropropionic acid mouse model of HD, where there is a clear mitochondrial defect, infusion of DβHB attenuated striatal lesion, microglia activation and motor deficit. The epigenetic effect of DβHB was also assessed in a genetic HD model, in which mutant huntingtin protein (mhtt) induces histone deacetylation. In stably transfected cells with inducible expression of mhtt or normal htt, DβHB completely prevented histone deacetylation via a mechanism independent of mitochondria and histone deacetylase inhibition. To assess the potential clinical relevance, DβHB was infused in transgenic R6/2 mice (which express ~150 CAG repeats), in which both mitochondrial dysfunction and histone deacetylation have been demonstrated. The data show that DβHB significantly extended life expectancy by ~30%. Due to the pharmacokinetic limitations of DβHB, a ketogenic diet is used to deliver high and sustained levels of DβHB to further maximize the neuroprotective effects of this ketone body. The present study suggests that DβHB and the ketogenic diet may represent novel treatments for HD by targeting both the mitochondrial and epigenetic abnormalities.

1721 BRAIN UPTAKE AND BEHAVIORAL CHANGES IN RODENTS FOLLOWING NEONATAL EXPOSURE TO THE CYANOBACTERIAL TOXIN BMAA (β-N-METHYLAMINO-L-ALANINE).

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The neurotoxin BMAA is produced by all major groups of ubiquitous cyanobacteria (blue-green algae). It is an agonist at both ionotropic and metabotropic glutamate receptors and implicated in the etiology of the progressive neurodegeneration found among the population in Guam. The neurotoxic potency of BMAA is relatively low in adult rodents. Brain growth spurt (BGS) is a sensitive period in development and appears in humans from the last trimester of gestation until about 3 years of age. In rodents is it a short period that peaks around postnatal day (PND) 10. The aim of this study was to examine the transfer and effects of BMAA during BGS in rodents.

Pregnant mice and 10-day-old mice pups were given an injection with 3H-BMAA and processed for tape-section autoradiography. The autoradiographic study revealed a transplacental transfer and an increased level in the fetal brain. The study also revealed a transfer of radioactivity to the neonatal brain with selective binding in the hippocampus, cerebellum, striatum and spinal cord. Furthermore, acute and possible long-term effects of BMAA were investigated in neonatal Wistar rats. At PND 9 and 10 three groups of neonatal rats were given a s.c. injection of 200, 600 mg/kg BMAA or vehicle. Acute effects on body weight gain were observed and two of the pups in the high exposure group showed emprosthotonic seizures and were sacrificed. At PND 13 locomotor ability was assessed in a modified open field. The results revealed locomotor disturbances in pups treated with the highest dose and also a dose-dependent increase in locomotor activity for BMAA-treated pups.

In conclusion, there was a transfer of 3H-BMAA to the fetal and neonatal mouse brain and significant cerebral effects were observed in neonatal rats given BMAA demonstrating that neonatal animals are susceptible to BMAA during the BGS period. The induction of permanent behavioral changes and neurobiological effects in adult animals following neonatal exposure to BMAA will also be studied.

1722 ASSESSMENT OF THE *IN VIVO* CONSEQUENCES OF PBDE EXPOSURE TO THE DEVELOPING VERTEBRATE UTILIZING THE ZEBRAFISH MODEL.

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Polybrominated diphenyl ether (PBDE) flame retardant concentrations are increasing in the environment, wildlife, and humans. There is concern that developing organisms are particularly susceptible to PBDE exposure, however little is known about the potential developmental toxicity of PBDEs. The zebrafish provides an unparalleled high-throughput in vivo model to assess the potential ramifications of PBDEs to developing vertebrates. The aim of these studies was to investigate the ramifications of PBDE developmental exposure using a suite of endpoints including behavioral impairments. Embryo and larval zebrafish were exposed to each of the major congeners found in biological tissues and environmental matrices (i.e. BDE 47, 49, 99 and 100). Embryos were exposed from either 5 hours post fertilization (hpf) to 120 hpf or 24 hpf to 120 hpf (dechorinated) at concentrations from 0.78 μM to 50 μM. We observed a number of effects to embryo morphology between 120 and 168 hpf at ≥ 3.13 uM in both exposure windows. All BDEs studied showed some lethality by 168 hpf at 12.5 μM. There was no developmental delay from exposure as indicated from hatching and other developmental milestones. BDE 47, 49 and 99 caused a significant dorsal curvature of the tail at 12.5 μM between 120 and 144 hpf. Only BDE 47 caused significant cardiac arrhythmias at 144 hpf between 6.25 μM and 12.5 μM. Larval animals exposed to BDE ≥ 6.25 μM exhibited a decreased touch and escape response by 120 hpf. Similar to reports in mammalian models all the BDEs studied caused noticeable behavioral impairment. While further study is needed, these data provide the framework toward the identification of conserved molecular mechanisms of PBDE developmental toxicity in vertebrates. Supported by NOAA #NA05NS4781253.

1723 DEVELOPMENTAL NEUROTOXICITY ASSESSMENT IN ZEBRAFISH: A SURVEY OF 200 ENVIRONMENTAL TOXICANTS.

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Conventional assessment of neurotoxicity in mammalian models relies on histological, neurophysiologic, and behavioral studies that are laborious and time consuming. The zebrafish (Danio rerio), is an increasingly attractive alternative model for developmental neurotoxicity, in part due to its embryonic transparency, rapid development, and simplicity of chemical delivery. Previous studies at Phylonix demonstrated a high correlation between developmental neurotoxicity observed in zebrafish with findings in mammalian models. In particular we characterized brain apoptosis and tail motor neuron defects that correlated with behavioral defects. To further establish the feasibility of a zebrafish model for developmental toxicity, we surveyed developmental neurotoxicity of 200 environmental toxicants chosen from the Voluntary Children's Chemical Evaluation Program (VCCEP) list and/or EPA Emergency Planning and Community Right-to-Know Act (EPCRA). We looked at the following parameters of neurotoxicity: 1) lethality, 2) brain apoptosis, 3) axon tract disruption in the brain and tail, 4) motor neuron formation in the tail, 5) catecholaminergic neurotoxicity, and 6) motility. From the 200 toxicants surveyed, we identified several compounds that showed effects on one or more of the parameters examined. Some compounds showed broad toxicity across all neuroanatomical endpoints. We also identified compounds that increased and decreased motor activity in day 6 zebrafish. Our results show that the zebrafish model has predictive value as an alternative model for developmental neurotoxicity screening. This work was supported by National Science Foundation SBIR award # 0548657.
(NOTE TO ABSTRACT REVIEWERS: A complete list of the 200 compounds used in this study will be included on the poster.)

1724 THE SEQUENTIAL UPREGULATION OF CELL CYCLE GENES AND MASH1 INCREASES THE PROLIFERATION AND DIFFERENTIATION OF LATE-BORN RETINAL PROGENITOR CELLS (RPC) IN GESTATIONALLY LEAD-EXPOSED (GLE) MICE.

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GLE produces a novel adult retinal phenotype characterized by a dose-dependent increase rod photoreceptors and bipolar cells: late-born RPCs. Our goal was to determine the mechanism of this unique phenotype. C57BL/6 mice were exposed to water or a lead drinking solution from two weeks prior to mating, throughout gestation and until postnatal day 10 (PN10) to model human equivalent GLE. Retinal RNA was extracted at embryonic day (E) 16.5, E18.5, postnatal day (PN) 2, PN6 and PN10. Affymetrix mouse genome arrays were run. Gene expression was validated by real time Q-PCR. Cluster analysis used Cluster and TreeView software. Gene Ontology (GO) analysis used NIH-DAVID. Confocal and Western blots examined the localization and amount of protein product, and regulation of RPC fate. Peak [BPb] was <1 and ~25 μg/dL. 4,899 genes were differentially expressed in control retinas from E16.5-PN10. Based on the level and temporal pattern of expression, we created 5 distinct clusters. GLE mice, relative to controls, had 410 genes upregulated and 452 genes downregulated. Cluster 4 genes that regulate RPC proliferation [e.g., Ccnd1, Mki67] were upregulated in GLE retinas starting at E18.5. Mash1, a cluster 4 gene and transcription factor required for the selective differentiation of rods and bipolar cells, was upregulated at PN6 and PN10 in GLE retinas. Confocal and immunoblots showed increased expression of cyclin D1 and Ki67 in developing GLE retinas. Mash1 was localized to post-mitotic RPCs and differentiating late-born neurons, and its concentration increased in GLE retinas. Thus, GLE sequentially upregulated cell cycle genes and Mash1, which produced a long-term selective increase in rods and bipolar cells. Moreover, these novel cellular and structural changes account for the increased rod-mediated ERG amplitude observed in GLE children, and adult monkeys and rodents. Supported by NIH Grants ES012482, EY07751, EY11115 and Sramek Foundation.

1725 NEW NEUROTOXICITY TEST WITH ZEBRAFISH EMBRYOS.

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Zebrafish embryo presents some advantages (fast development, transparency, small size, etc.) that allow a fast and effective visualization of the correct/incorrect development of those morphologic and functional details implied in acute or organ-specific toxicity.
Based in its previous experience in the development of acute toxicity and teratogenic tests, ZF Biolabs, in collaboration with the "Canal de Isabel II" (Madrid), has developed a new neurotoxicity test with zebrafish embryos in their early stages of development.
This test is based on morphological (CNS, PNS) and functional (spontaneous movement) endpoints (Kimmel et al, 1974; Nagel, 2002). Observation of neurotoxic effects in the embryo is carried out at 28 hpf (hours post-fertilization) by in vivo optical microscopy, after an immersion of 24 hours in the toxic solution to test. Embryos used for the test have to pass a double quality control selection at 3-4 hpf. The test has been validated with the following compounds: Acetone, Caffein, Colchicine, Hydroxyurea, Isoniazide, Valproic Acid, Methyl-Mercuric Chloride, Urethane, Diphenidramine and Aspirine, obtaining results of LC50, EC50neurotoxicity and establishing the relation between both values in an index and a graphical representation that define the presence/absence of neurotoxic potential, or the doubtful status of each compound.
Results obtained in the study of above cited compounds allow contemplating this test as a valid alternative to determinate the neurotoxic potential of a big group of chemical substances. Also, an "Atlas of Neurotoxicity" has been elaborated, which contains the experimental protocol and textual and graphical definitions of all endpoints for their correct interpretation by any qualified observer.

1726 EFFECT OF GABA AGONISTS ON KETAMINE-INDUCED NEUROTOXICITY IN PND-3 MONKEY FRONTAL CORTICAL CULTURES.

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Previous data suggest that exposure of neonatal rats to various anesthetics, including NMDA antagonists, triggers widespread apoptotic neurodegeneration during a critical phase of brain development. Monkey cortical cultures were treated for 24 hrs with 1, 10 or 20 μM ketamine alone; 1, 5, 10 or 50 μM midazolam alone; 10, 50, 100 or 500 μM baclofen alone; and ketamine (10 μM) plus midazolam or baclofen at various doses. After washout of ketamine, midazolam and baclofen, cultures were kept in serum-containing medium (in the presence of glutamate) for 24 hrs. Ketamine (10 μM) produced a substantial increase in the release of LDH into the culture medium and a reduction in mitochondrial metabolism of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide dye (MTT, for cell survival). No significant neurotoxicity was observed in midazolam (1, 5, 10 and 50 μM)-treated cultures. No enhanced neuronal cell death was detected in baclofen (all 4 concentrations)-treated cultures. Co-incubation of 10 μM midazolam with ketamine (10 μM) partially prevented ketamine-induced neuronal cell death. Co-administration of 100 (or 500) μM baclofen with ketamine almost completely pro-

tected neuronal cells from ketamine-induced damage. These results indicate that ketamine administration causes a dose-dependent increase in neurotoxicity in perinatal monkey cortical cultures. Co-administration of midazolam or baclofen prevented this enhanced neurotoxicity caused by ketamine, indicating that the GABA and glutamatergic systems could be co-activated during physiological and pathological patterns of activity, and stimulation of the GABA system could inhibit the activation of NMDA receptors. These data suggest that combinations of NMDA antagonists with GABA agonists, especially GABAB agonists, may reduce the risks associated with the use of NMDA antagonists during development.

1727 VOLTAGE-DEPENDENT SODIUM CHANNEL GATING MODIFIER TOXINS PRODUCE SODIUM INFLUX IN NEOCORTICAL NEURONS.

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Voltage-dependent sodium channels (VDSC) play an essential role in the initiation and propagation of action potentials in neurons. The essential properties of VDSC are activation, inactivation and Na^+ ion permeation. Naturally occurring and synthetic neurotoxins have been identified that modify each of these VDSC functions. Using murine neocortical neurons in primary culture, we have compared the ability of an array of VDSC gating modifiers to trigger Na^+influx. Intracellular sodium concentration ($[Na^+]_i$) was monitored using the Na^+ indicator dye SBFI. The basal $[Na^+]_i$ in DIV 9 neocortical neurons was found to be 10.0 ± 0.5 mM. An array of VDSC gating modifiers including brevetoxin (PbTx-2), antillatoxin, veratridine and deltamethrin all produced a concentration-dependent increase in $[Na^+]_i$. The EC_{50} values for PbTx-2 (106.3 nM), antillatoxin (89.7 nM), veratridine (908 nM) and deltamethrin (942 nM) were consonant with previously reported potencies for these toxins. The efficacies of antillatoxin and PbTx-2 were similar, but significantly greater than those observed with veratridine and deltamethrin. Both antillatoxin and PbTx-2 produced maximum increments of $[Na^+]_i$ of greater than 40 mM in neocortical neurons. Inasmuch as the PbTx-2 site and the pyrethroid binding site on VDSCs are allosterically coupled, we assessed the ability of a low PbTx-2 concentration to enhance deltamethrin-induced increase in $[Na^+]_i$. Although inactive alone, 10 nM PbTx-2 produced a significant enhancement of deltamethrin-induced elevation of $[Na^+]_i$. These data indicate that the Na^+-sensitive fluorescent dye SBFI can be used to quantitatively compare gating modifier toxin-induced sodium influx in neurons. Given the potential role of Na^+ as a signaling cation, this approach will moreover be useful in characterizing the influence of gating modifier toxins on neuronal signaling. (Supported by NIH grant NS053398 to W.H.G. and T.F.M.)

1728 THE ROLE OF NMDA RECEPTOR SUBUNITS IN PHENCYCLIDINE (PCP)-INDUCED NEURONAL APOPTOSIS IN RATS.

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Phencyclidine (PCP) is an N-methyl-D-aspartate (NMDA) receptor antagonist known to cause selective neurotoxicity in the cortex, although the exact mechanism by which it does so remains unknown. The purpose of this study was to determine whether up-regulation of the NR1, NR2A, and/or NR2B subunits of the NMDA receptor is associated with PCP-induced apoptotic cell death. A corticostriatal organotypic slice culture preparation from postnatal day 7 Sprague-Dawley rats was used to determine the effects of antisense oligonucleotides on PCP-induced apoptosis and NR subunit upregulation. NR1, NR2A, or NR2B antisense strands (2 μM) were added alone or with PCP (3 μM) to the culture preparation for a 48-hr incubation period. Enhanced cortical apoptosis, measured using an ELISA assay for apoptotic cell death, was seen in the PCP-alone samples, as well as in the NR2B antisense plus PCP samples. Co-incubation of PCP with either NR1 or NR2A antisense significantly reduced PCP-induced apoptosis. Western analysis of treated cortex was used to measure levels of the pro-apoptotic protein, Bax, and showed that both NR1 and NR2A antisense attenuated the PCP-induced increase in Bax. PCP caused no significant apoptosis in the striatum. These data suggest that NR1 and NR2A antisense both provide some degree of neuroprotection from PCP-induced apoptosis and that upregulation of the NR1 and/or NR2A subunits following PCP administration is at least partly responsible for the observed apoptotic DNA fragmentation. Supported by NTP and NCTR Experiment #E-2155

1729 DESIGN CONSIDERATIONS AND IMPLICATIONS FOR NEUROBEHAVIORAL DATA.

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In the US EPA 870.6300 guideline for developmental neurotoxicity studies, the ages for assessment of preweaning locomotor activity testing are defined to be PND 13, 17 and 21 and auditory startle response testing is required to be conducted around the time of weaning. To minimize variability in the data, strict controls over the testing procedure are required. These controls include counterbalancing of groups within test sessions, consistency in the times of day that tests are performed, similarity of ages to be tested and appropriate representation of litters within the groups. However, guideline requirements specify exact ages for testing, and given the inability to control breeding and subsequent parturition within these studies, the number of days of testing and the length of time required to complete testing on each particular day for a given interval can vary within and across studies. In addition, growth rates of offspring can vary within and across study groups, and these differences in growth are not always treatment-related. It was recently hypothesized that these factors can significantly impact the overall variability of the endpoints being tested. An analysis of historical control data from this laboratory revealed that the time of day of testing can have a significant influence on the maximal startle response exhibited by the test subjects. For example, male subjects that were tested earlier in the day generally demonstrated greater responsiveness to the startle stimulus than those tested later in the day. Given the expectations of the EPA DNT guideline regarding selection of animals for future generations, evaluation of animals in neurobehavioral test batteries and specified ages at testing, variability within the data should be defined even under the most strictly controlled conditions.

1730 OXIDATIVE DNA DAMAGE AND OCCUPATIONAL EXPOSURE TO PAH.

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Biomarkers of exposure and effect were used to study oxidative DNA damage in 171 PAH-exposed workers of coke, carbon electrode, refractory material and steel production. 48 construction workers served as referents. Personal air monitoring of 16 EPA-PAHs was carried out. 1-hydroxypyrene (1-OHP) and 1-,2-,3-,4+9 hydroxyphenanthrenes (ΣOHPhe) in urine and 8-oxodGuo and DNA strand breaks in blood were measured post shift. Biomarker levels were adjusted for smoking, age and other factors. Median concentration of 16 EPA-PAH was 30.2 $\mu g/m^3$. 1-OHP and ΣOHPhe in urine were significantly higher in exposed workers (7.8 and 12.4 μg/g crea) than in referents (0.18 and 1.0 μg/g). Airborne pyrene and phenanthrene was associated with 1-OHP (r_S=0.52, P<0.0001) and ΣOHPhe (r_S=0.73, P<0.0001). However, calculating the relative internal exposure for each worker (internal dose devided by external dose) our results also pointed to considerable skin absorption of PAH. Internal exposures were in the order "steel" > "carbon electrode" ≈ "refractories" > "coke production" > "referents". Significant increased median levels were observed for exposed workers compared to referents for 8-oxodGuo (5.7 vs. 2.3 adducts/10^6 nucleotides) and DNA strand breaks (Olive Tail Moment: 2.3 vs. 1.3). However, a dose-dependent increase of DNA damage with increasing exposure to PAH in terms of a statistical association could not be observed. Overall, our study in PAH-exposed workers shows that biomarkers of exposure such as 1-OHP and ΣOHPhe rather than personal ambient monitoring should be used for exposure assessment due to potential dermal absorption of PAH. Our results also provide evidence that in workers of coke, refractory material, carbon electrode and steel production increased oxidative DNA damage can be found. However, PAH exposure and PAH-induced redox-cycling *in-vivo* cannot be made responsible for the observed increase in oxidative DNA damage because of the missing statistical association between both, biomarkers of exposure and oxidative DNA damage.

1731 BIOMARKERS OF PAH EXPOSURE AND GENOTOXIC EFFECTS IN HUMAN POPULATIONS.

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Polycyclic aromatic hydrocarbons (PAHs) are one of the most common classes of environmental contaminants. Humans may be exposed to these compounds in air, food, soil and water. Individual PAHs have been clearly linked to cancer in animal studies, and PAH exposure in occupational settings has also been linked to some cancers. The goal of the current study is to investigate biomarkers of exposure and genotoxic effects in an urban and a rural population in Azerbaijan. The urban population in Azerbaijan was selected as they live in close proximity to several petrochemical facilities. In addition, the World Health Organization (WHO, 1999) determined that Sumgayit, Azerbaijan has an elevated rate of birth defects. In most

cases, samples were available from three generations in each family. Exposures have been estimated from dust sampling in homes, as well as measurement of PAHs in plasma and 1-hydroxypyrene in urine. Biomarkers of genetic effects include bulky DNA adducts and Sister Chromatid Exchange in lymphocytes. Median PAH concentrations in plasma were significantly higher in men and women from the urban population (223 ng/mL) when compared to the rural population (96 ng/mL). The maximum level of PAHs detected in plasma from the rural population was 172 ng/mL, while the maximum observed in the urban population was 946 ng/mL. Significantly higher levels of DNA adducts were also observed in the urban population; and, men from the rural population were observed to have significantly higher levels of adducts than women (presumably due to smoking). However, there was no significant difference in adduct levels in men and women from the urban population. The range of SCEs in lymphocytes from four families was 8.3-11.7, while the normal range is 4.3-7. The data confirm that PAH concentrations in dust from the selected urban houses is elevated. In addition, families in the urban population exhibit biomarkers of exposure and early genotoxic effect.

1732 BIOMONITORING OF EXPOSURE TO POLYCYCLIC AROMATIC HYDROCARBONS IN INDIVIDUALS LIVING NEAR AN ALUMINUM PLANT IN QUEBEC, CANADA.

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A biomonitoring study was conducted to assess the importance of exposure to polycyclic aromatic hydrocarbons (PAHs) in non-occupationally exposed non-smoking adults living in the vicinity of an aluminum plant. Metabolites of several PAHs (pyrene, naphthalene, chrysene, fluoranthene, benz(a)anthracene) were measured in the urine of the participants, including 1-hydroxypyrene (1-OHP) as a validated biomarker and pyrene-diones as novel biomarkers. A total of 53 to 56 individuals living about 1 km from the plant were repeatedly compared to 50 to 56 individuals living at least 11 km from the plant. Urine samples were collected on different days and weeks and were analyzed by HPLC-MS-TOF. For most sampling days, individuals living near the plant showed slight but significantly higher excretion values of 1-OHP and pyrene-diones (on average up to 1.5 and 2.4 times higher, respectively) than individuals living further from the plant. In the group living near the plant, geometric mean concentrations of 1-OHP varied from 0.047 to 0.058 µmol/mol creatinine, depending on the sampling day, as compared to 0.035 to 0.040 µmol/mol creatinine in the reference group. Corresponding mean values for pyrene diones were 0.032-0.056 µmol/mol creatinine and 0.023-0.039 µmol/mol creatinine, respectively. Urinary 1- and 2-naphthols were also measured as a reference and showed no significant difference between the two groups except for two sampling days where a significantly higher excretion of 1-naphthol was observed in controls; metabolite concentrations of the other monitored PAHs (chrysene, fluoranthene, benz(a)anthracene) were mostly below the analytical limit of detection of 0.005 to 0.01 µg/L depending on the metabolite. Results show that individuals living near the aluminum plant were repeatedly exposed to higher pyrene exposure levels. However, uptake of the other monitored PAHs due to the plant was too small to significantly increase the excretion of their metabolites.

1733 BLOOD URANIUM (U) ISOTOPIC ANALYSIS AS A MEASURE OF DU EXPOSURE IN U.S. SOLDIERS.

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Urine uranium (U) excretion has become the accepted means by which to monitor elevated exposures to U, both in occupational cohorts and environmentally exposed populations. In addition, specific exposure to depleted uranium (DU), as experienced by U.S. soldiers in combat scenarios involving DU munitions, has been made possible by refined methods of analysis that allow the detection of U^{235}/U^{238} ratios in urine samples with concentrations of U that lie within the normal range of urine U concentrations. Blood concentrations of U are also elevated in DU exposed soldiers, and the recent development of an ICP-MS technique with sufficient sensitivity to measure U^{235}/U^{238} ratios in blood samples at total U concentrations as low as 50 ppt provides the opportunity to determine whether blood could also be used for detecting DU exposure in U.S. soldiers. Blood U concentrations in Gulf War soldiers exposed to DU by inhalation, ingestion and/or embedded fragments ranged from 0.01 to 0.80 µg/L in 2007. Four of the 33 soldiers tested had blood U concentrations above the average concentration reported for non-occupationally exposed New York City residents (0.14 µg U/kg wet weight) (Fisene, IM and Perry, PM, Hlth Phys 49: 1271, 1985). Isotopic analysis of blood samples from the Gulf War cohort identified 5 soldiers out of 33 with an isotope signature consistent with DU exposure, while analysis of urine samples from these same individuals identified 10 soldiers with DU present in their urines. Thus, urine remains the best matrix for detecting exposure to DU in U.S. soldiers. *Supported by the Department of Veterans Affairs.*

1734 HUMAN EXPOSURE TO BISPHENOL A BY BIOMONITORING: METHODS, RESULTS AND ASSESSMENT OF ENVIRONMENTAL EXPOSURES.

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Human exposure to bisphenol A is controversially discussed due to reported low dose effects of bisphenol A on some reproductive parameters in rodents. This review critically assesses published methods for monitoring of bisphenol A concentrations in human blood and urine samples and the reported concentrations of bisphenol A in blood and urine of non-occupationally ("environmentally") exposed humans. From the many methods published to determine bisphenol A concentrations in biological media, mass spectrometry-based methods are considered most appropriate due to high sensitivity, selectivity and precision. Moreover, a number of factors interfering with the analytical determination of bisphenol A in the low concentrations expected in biological fluids were identified influencing precision and interpretation of the obtained data. In human blood, based on the known toxicokinetics of bisphenol A in humans, the expected concentrations of parent bisphenol A are very low due to rapid biotransformation of bisphenol A to bisphenol A-glucuronide, which is devoid of hormonal activity. Moreover, the very rapid clearance of the major metabolite, bisphenol A-glucuronide, from blood results in severe limitations in the use of blood levels of bisphenol A for assessment of population exposures. Due to the rapid and complete excretion of orally administered bisphenol A with urine in humans within less than 24 hours, urine is considered as appropriate for bisphenol A exposure assessment. In "spot" urine samples from several cohorts, bisphenol A (as glucuronide) was present in average concentrations in the range of 1 - 3 µg/L suggesting that daily human exposure to bisphenol A is below 6 µg per person (< 0.1 µg/kg bw/day) for the majority of the population. These doses are well below the recently allocated tolerable intake (TDI) for bisphenol A and also below the doses which have been reported to result in changes in reproductive parameters in rodents administered bisphenol A.

1735 THE ROLE OF ESTROGEN METABOLISM IN THE INITIATION OF BREAST CANCER: DETECTION OF POTENTIAL EARLY BIOMARKERS FROM A CASE-CONTROL STUDY.

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Prolonged exposure to high estrogen levels in women has been linked to increased incidence of breast cancer. However, the mechanism by which the estrogens estrone (E1) and estradiol (E2) play a role in the initiation of breast cancer is still in controversial. We hypothesize that E1 and E2 are metabolized to reactive catechol estrogen quinones [E1(E2)-2,3-Q and E1(E2)-3,4-Q], which can react with glutathione and DNA, or be reduced to catechol estrogens. In particular, quinones can react with DNA to form depurinating 4-OHE1 (E2) - N7Gua and 4-OHE1 (E2) - N3Ade adducts. Apurinic sites that are formed by depurination can induce mutations leading to cancer. Once released from DNA, the depurinating estrogen-DNA adducts are shed from cells into the bloodstream. Hence, by analyzing the estrogen metabolite profiles in the serum, potential biomarkers of breast cancer can be detected.

The purpose of this case-control study is to detect and identify a potential early biomarker of breast cancer. Serum samples from 47 Caucasian women without breast cancer (controls), 37 Caucasian women with breast carcinoma (cases) and 53 Caucasian women considered to be at high risk of breast cancer (Gail scale > 1.66%) were analyzed by ultra-performance liquid chromatography-electrospray ionization mass spectrometry. Results show that the ratio of DNA adducts to the sum of the corresponding E1/E2 metabolites and conjugates is significantly higher in cases and high-risk group compared to controls ($p<0.01$). There is no significant difference between cases and high-risk women.

This study suggests that the above ratio could serve as an early biomarker to predict risk of breast cancer. It could also be a useful tool for evaluating the role of environmental estrogens in the risk of breast cancer and for developing breast cancer prevention strategies.

1736 ANALYSIS OF 1, 2;3, 4 DIEPOXYBUTANE SPECIFIC PROTEIN ADDUCT IN OCCUPATIONALLY EXPOSED WORKERS, PART 2.

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Butadiene (BD) is an industrial chemical used in the production of synthetic rubber and is also found in gasoline and cigarette smoke. It is a multisite carcinogen in rodents, with mice being more susceptible then rats. BD is categorized by NTP and

US EPA as carcinogenic to humans by inhalation. In vivo BD is metabolized to several epoxides that differ in their mutagenic potency by up to 200-fold and form DNA and protein adducts. To advance our understanding in BD metabolism and carcinogenesis, it is important to have accurate measure for the internal formation of the individual epoxides. Therefore the formation of the promutagenic 1,2;3,4-diepoxybutane (DEB) in vivo was determined by analysis of the corresponding protein adduct, N,N-(2,3-dihydroxy-1,4-butadiyl)-valine (pyr-Val). For this purpose, globin samples from BD-styrene polymerization plant workers in the Czech Republic (from the so called "Albertini-1 study") were analyzed for pyr-Val. In brief, stable isotope internal standard was added to 50 mg globin, followed by trypsin digestion, purification by immunoaffinity (IA) chromatography and quantification by nano-LC-MS/MS (LOD and LOQ at 1 and 4 fmol, respectively). Among the analyzed 81 subjects from the Czech study: 23 controls, 24 monomer and 34 polymerization workers with mean exposures of 0.010, 0.292 and 0.814 ppm BD, respectively, 68 samples had quantifiable amounts of pyr-Val ranging from 0.079 to 0.859 pmol/g globin. The amount of pyr-Val was significantly higher in the polymerization workers than in the monomer workers and controls. Combining the data from all groups, there was a significant correlation with BD exposure. Also pyr-Val was significantly correlated with the other BD derived protein adducts. No significant association between current smoking status and pyr-Val was found.

1737 A PILOT STUDY COMPARING BIOMARKERS OF EXPOSURE IN SMOKERS OF MENTHOL AND NON-MENTHOL CIGARETTES.

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Mentholation of cigarettes has been speculated to influence smokers' exposures to smoke constituents, although a number of extant epidemiological studies suggest that the risks of smoking mentholated and non-mentholated cigarettes are similar. A parallel-arm study of 120 male and female smokers of "Lights" cigarettes (9-10 mg FTC "tar") was performed to determine whether menthol and non-menthol cigarette smoking results in differences in smoke constituent exposure biomarkers from cigarettes having similar (+/- 20%) FTC smoke yields. Nominal cigarette smoke yields (FTC "tar"/nicotine/CO), subjects' sex and demographic characteristics, and designated cigarette brands were balanced to the extent practical in order to assess any independent effects of cigarette mentholation. Cigarette consumption was documented by filter butt collections at enrollment and on the days before two 24-hour study intervals spaced one week apart. Blood carboxyhemoglobin levels were measured at mid-afternoon at the beginning and end of the two 24-hour urine collection periods. Urinary nicotine equivalents (nicotine, cotinine, trans 3'-hydroxycotinine and glucuronides) were determined as a measure of nicotine exposure; urinary 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronide, were quantified to assess exposure to the tobacco- specific nitrosamine NNK (4-(N-nitrosomethylamino)-1-(3-pyridinyl)-1-butanone). A preliminary examination of urinary metabolite data suggested that neither median urinary nicotine equivalents nor total NNK metabolites were increased among smokers of mentholated cigarettes. Median blood carboxyhemoglobin values did not indicate a substantive difference in exposure between the cigarette types. Ad libitum smoking of mentholated and non-mentholated "Lights" cigarettes resulted in similar levels of excreted biomarkers of smoke exposure. The present results do not indicate increased smoking intensity or exposure among smokers who prefer menthol cigarettes relative to those smoking non-mentholated cigarettes.

1738 BIOMARKER IDENTIFICATION AND EXPOSURE ESTIMATE FOR PHTHALATES IN A PREGNANT POPULATION.

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Phthalates are known reproductive and developmental toxicants in experimental animals, but there are limited data implicating phthalate exposure with adverse health effects in humans, especially pregnant women and newborns. To identify the biomarkers for exposure assessment, this study measured the phthalate metabolites in the maternal urine, maternal blood, cord blood, and amniotic fluid samples in 150 pregnant women from central New Jersey. Most monoesters in maternal urines were comparable to, or lower than the levels in the US general and female population (CDC 3rd Reports of NHANES 2001 – 2002 data), except for mono- (2-ethylhexyl) phthalate (MEHP), mono- (2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), and mono- (2-ethyl-5-oxohexyl) phthalate (MEOHP). The median values of MEHHP (105 μg/L) and MEOHP (95.1 μg/L) are more than 5 times the levels in the CDC Report, while the median value of MEHP over 20 times higher. This high level of MEHP may indicate a recent exposure to the parent chemical di-2-ethylhexyl phthalate (DEHP). Significantly higher level in maternal urine than in serum suggest the urinary metabolites be more reliable biomarkers of exposure. Calculation of the daily intakes using their urinary biomarkers indicated about 25% of the pregnant women had exposures to DEHP higher than the EPA reference dose (RfD 20 μg/kg/day), and some 5% were higher than EU total daily intake (TDI 40 μg/kg/day). Data indicates that the urinary metabolites, essentially the monoesters for low MW phthalates (DEP, DBP, BBzP), and the oxidative metabolites for high MW phthalates (DEHP, DnOP), are reliable biomarkers for exposure assessment. The relatively high level of exposure to DEHP in this study population call for further research to explore if the prenatal exposure would lead to any adverse effects on fetal development.

1739 PSTC VXDS#5 KIDNEY INJURY MOLECULE-1: A SENSITIVE AND SPECIFIC BIOMARKER IN VARIOUS FORMS OF ACUTE KIDNEY INJURY IN HUMANS.

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The goal of our ongoing studies has been to identify and qualify biomarkers that are sensitive, specific, and predictive of outcome in acute kidney injury (AKI). We first compared the performance of kidney injury molecule-1 (KIM-1) with eight promising urinary biomarkers in a cross sectional study of 102 adult patients with clinically established AKI compared to 102 patients (50 healthy controls, 13 ICU patients, 39 pre-cardiac catheterization) without AKI. KIM-1 was found to have the highest sensitivity and specificity amongst all nine biomarkers tested [neutrophil gelatinase associated lipocalin (NGAL), interleukin-18 (IL-18), hepatocyte growth factor (HGF), cystatin C (Cys), N-acetyl-β-D-glucosaminidase (NAG), vascular endothelial growth factor (VEGF), chemokine interferon-inducible protein 10 (IP-10; CXCL10) and total protein. In studies of nephrotoxicity, KIM-1 and NAG were found to increase prior to changes in serum creatinine (SCr) in patients administered gentamicin or cisplatin. In another study urine was collected before and 4h after cardiac catheterization in 64 patients receiving radiocontrast, and in subsets at 24h (N = 48) and 48h (N = 26). Six patients had a greater than 25% or 0.5 mg/dL increase in SCr within 48h of the procedure. We found no difference in any of 4 urinary biomarkers in patients with and without an increase in SCr, and no correlation between the volume of radiocontrast and biomarker levels. This preliminary study of patients undergoing cardiac catheterization demonstrates a discrepancy between kidney tubular injury as reflected by urinary biomarkers and the standard measure of contrast nephropathy, SCr. In renal transplant and native kidney biopsies from patients with various primary renal diseases, tissue KIM-1 staining of proximal tubules correlated very well with histological injury and functional significance as determined by SCr. In summary, KIM-1 appears to be highly sensitive and specific biomarker for early detection of acute kidney injury, more sensitive than SCr.

1740 EFFECT OF PCB CONTAMINATED SOILS ON SERUM PCB LEVELS IN TRAILER PARK RESIDENTS.

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Exposure to soils contaminated with polychlorinated biphenyls (PCBs) can potentially contribute to the body burden of these compounds as a result of inadvertent ingestion, dermal penetration, and inhalation of suspended soil and/or impacted house dust. Elevated soil PCB levels of up to approximately 700 ppm were found at several homes located in a California trailer park. The short-term response included covering of impacted exposed soils with asphalt. Residents were provided the opportunity to participate in a voluntary PCB blood testing program. A total of 118 volunteers (91 adults and 27 children; 23 and 9 from impacted residences, respectively) provided blood samples for total PCB and blood lipid measurements. Demographic and other information that might influence total serum PCB concentrations were obtained through questionnaires. Mean serum PCB concentrations for persons residing in all areas were similar to mean concentrations reported for the general population. Mean serum PCB concentrations in adults from the impacted (1.2 ppb, SD 1.0) and non-impacted (1.0 ppb, SD 1.3) areas were not significantly different. No significant differences were found between children residing in impacted (0.30 ppb, SD 0.44) and non-impacted (0.39 ppb, SD 0.61) areas. Serum total PCB was positively correlated with increasing age ($p<0.0001$) and with estimated total serum lipid concentrations ($p=0.02$). No correlation was found between total measured serum PCB and house dust (floor or carpet) PCB levels for residents of homes that underwent interior testing ($r= -0.15$, $p=0.42$ for all, $p=0.51$ for adults, $p=0.65$ for children). Consequently, soil contamination did not appear

to have impacted serum PCB concentrations in these residents (p=0.77). The findings of this study add to data from other investigations of PCB-contaminated sites that have not demonstrated significant correlations between contaminated soils and serum levels.

1741 EVALUATION OF DIOXIN-LIKE COMPOUNDS IN WORKERS FROM A PRIMARY MAGNESIUM PRODUCTION FACILITY RELATIVE TO LEVELS OBSERVED IN THE GENERAL U.S. POPULATION.

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In 2004, the U.S. National Institute of Occupational Safety and Health (NIOSH) conducted a Health Hazard Evaluation (HHE) at a primary magnesium production facility. A series of biomonitoring data were collected as part of the HHE including measurements of dioxin-like compounds (DLCs) in blood of 30 workers believed to have the greatest potential for exposure. The goal of this study was to determine if the levels of DLCs in the workers were similar to general population or were elevated and suggestive of potential exposures at the facility. The first step involved calculating lipid-adjusted DLC toxic equivalency quotients (TEQs) for each facility worker. Next, DLC reference values were calculated using levels of DLCs measured in National Health and Nutrition Examination Survey (NHANES) participants of the same age and gender. The levels in the facility workers were compared to the applicable reference values. Results indicate that the majority of facility workers evaluated by NIOSH were below the 95th percentile of the reference population. Seven of the workers had levels which exceeded the 95th percentile and none were above the maximum value determined for male NHANES participants aged 40-59 years. This evaluation of the levels of DLCs in facility workers from a primary magnesium production facility thought to have the greatest potential for exposure indicates that site-related exposures to DLCs do not appear to have resulted in increased body burdens of DLCs in these workers when evaluated on a TEQ basis.

1742 IDENTIFICATION OF BIOMARKERS IN ALCOHOLIC LIVER DISEASE AND NON ALCOHOLIC STEATOHEPATITIS: PROTEIN EXPRESSION STUDIES.

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Alcoholic liver disease (ALD) and nonalcoholic steatohepatitis (NASH) are major health problems and cause high morbidity and mortality. Both diseases culminate in severe inflammation, fibrosis and cirrhosis. Preventive measures are generally hampered due to the lack of specific and sensitive markers for ALD and NASH. Therefore, present study was undertaken to develop discriminatory protein biomarkers that can predict ALD and differentiate it from NASH. Using strict clinical criteria, we collected plasma samples from adult male patients with ALD and NASH, and from healthy controls with no known disease. The plasma samples were subjected to 2-D gel electrophoresis and determined the proteins differentially expressed (±2 fold or greater) in patients with ALD vs. control (group I), NASH vs. control (group II) and NASH vs. ALD (group III). The proteins were identified by MALDI TOF/TOF followed by a database search. In group I, complement C3, complement C6, Ig gamma-3 chain and serum amyloid A-4 protein were down-regulated. The only protein that was differentially expressed (up-regulated) in group II was haptoglobin. In group III, complement C3, C4-B and C6, and hemoglobin were up-regulated, while, immunoglobulin J and Leucin-rich-alpha-2 glycoprotein down-regulated. Down regulation of complement activation system and immune function may be the prime target in ALD as compared to that in NASH. Therefore, complement C3, C4-B and C6, and hemoglobin and immunoglobulin J and Leucin-rich-alpha-2 glycoprotein could serve as ideal biomarker candidates for ALD in comparison to NASH. However, these findings need to be confirmed in a larger number of patients with ALD and NASH.

1743 PROTEOMIC BIOMARKER DISCOVERY BY PARTIAL LEAST SQUARES DISCRIMINANT ANALYSIS.

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An important application of proteomic data is classification of biological samples from at least two known phenotype populations. The differential features in proteomic profiles among phenotype populations can be further investigated as potential biomarkers for the underlying complex biological processes. We demonstrate, by example, the utility of PLS-DA to discriminate phenotype by proteomic profiling of blood plasma. The dataset consists of 38 male and female with either slow or rapid progression of chronic obstructive pulmonary disease (COPD) selected from a larger group of participants in a multi-center lung health clinical trial. There were 20 (6 female, 14 male) subjects with rapid progression and 18 (5 female,13 male) subjects with slow progression of COPD for a total of 38 plasma samples. The twelve most abundant plasma proteins were depleted using GenWay Seppro 12 spin columns, protein from the depleted samples was digested with trypsin and analyzed in duplicate using capillary LC-FTICR-MS. Analysis of the MS experiments was performed using in-house software tools to identify MS features, deisotope, normalize elution times, and match features to peptides, which were identified using an AMT tag strategy. The abundances of the individual peptides were computed by summing the intensity of the ions from a single scan or multiple scans that matched each peptide. The PLS-DA model of the lung health study data was developed using 2086 peptides and provided 267 statistically interesting peptides. Five components were significant such that 63% of the variation in peptide abundance explained 99% of the variation in phenotype. Additionally, a simple ad-hoc procedure to handling missing values is presented. In conclusion, we have shown PLS-DA with variable selection to be a practical, robust and useful multivariate data analysis method for the discrimination of phenotype and selection of possible biomarkers through proteomic profiling. Supported by ES16015 to PNNL, a multi-program national laboratory operated by Battelle for the U.S. DOE under Contract DE-AC05-76RL01830

1744 ASSESSMENT OF AUTOANTIBODIES TO NERVOUS SYSTEM PROTEINS FOLLOWING CHRONIC EXPOSURE TO BENZENE.

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It has been shown that autoantibodies to nervous system proteins may occur following exposure to heavy metals, pesticides, or organic solvents. Although benzene is known to be neurotoxic, studies on its neurotoxic potential following chronic exposure remain extremely scarce. A few reports indicate that occupational exposure to benzene results in increase of autoantibodies to several proteins in the nervous system. This study was initiated to assess whether serum autoantibodies to the neurofilament triplet (NF) and glial fibrillary acidic protein (GFAP) may be used as biomarkers of chronic occupational exposure to benzene. These two neural proteins were measured by ELISA and urinary phenol determined by gas chromatography with flame ionization detector in 34 petroleum refinery workers (mean age: 35 ± 15 years, mean duration exposure: 10 ± 3 years, mean ambient benzene level: 31 mg/m3 ± 5). The results were compared to 40 age- and sex-matched construction workers who served as controls. The mean urinary phenol in benzene-exposed subjects (72 ± 5 μg/g creatinine) was 8 times higher than in controls ($p = 0.0002$). Autoantibodies (IgG and IgM) to NF and GFA proteins were present in 85% and 71% of the benzene group, compared to 8% and 10% controls, respectively. Antibody titers were significantly higher in the former group ($p = 0.007$). A similar trend was observed for GFAP both in the proportion of subjects with antibodies and the titers. These results indicate that autoantibodies to neural proteins may be used as biomarkers in monitoring the neurotoxic effects of chronic exposure to benzene.

1745 BACKGROUND LEVELS OF HYDROGEN CYANIDE IN BREATH.

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An important source of intoxication by hydrogen cyanide (HCN) is exposure to fire gases. Rapid diagnosis and treatment is essential to avoid fatalities. One way of fast diagnosis might be via measurement of HCN in exhaled air (Johanson et al., SOT Annual Meeting, 2005). The aim of this study was to measure background levels of HCN in breath in healthy subjects. Such data are needed to distinguish normal breath levels from the elevated ones presumably generated in HCN intoxicated fire victims. Only a few studies with a limited number of subjects have been published (Boxer and Rickards 1952, Lundquist et al. 1988, Španěl et al. 2007).
In our study, background levels of HCN were measured by cavity ringdown spectroscopy (CRDS) in breath samples from 40 healthy human subjects (26 males, 14 females, age 21-61).
HCN was detected in the ppb range with a detection limit of about 1 ppb in breath. A preliminary analysis of the recordings suggest a median HCN level of 2.8 ppb (range ND-15 ppb, n=40). This range is well below the expected level of several hundred ppb expected in intoxicated subjects, according to a toxicokinetic-toxicodynamic model (Johanson et al., SOT Annual Meeting, 2005). No correlations

with smoking habits, recent meals, age or gender were seen. CRDS is very useful in measurements of HCN and other biomarkers present in breath in small quantities. The CRDS method has previously not been used to measure HCN in breath.
The study was supported by the Swedish National Board of Health and Welfare and the Academy of Finland.

1746 THE STABILITY OF THE OXIDATIVE STRESS MARKER, URINARY 8-HYDROXY-2'- DEOXYGUANOSINE (8-OHDG).

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Objectives: We examined the stabilities of urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) stored at room temperature (25°C) for 24 hours or at -80°C for 800 days.
Methods: Subjects were 19 males and 17 females, aged 23-58 years for 24 hours study or 9 males and 4 females, aged 24-54 years for 800 days study. We obtained information on the subjects by questionnaires and interviews. The level of urinary 8-OHdG was measured by HPLC using two-step separations. This study design followed the IRB protocol and obtained approval of the committee on research ethics at the institute the authors belong.
Results and conclusions: There were no significant changes of amount of urinary 8-OHdG by either storage conditions. We conclude that urinary sample can be stored at 25°C and below for 24 hours, when the research purpose included the determination of urinary 8-OHdG level. It was also stable for over two years period when stored at -80°C. Moreover, it was suggested that smoking habits impaired the stability and elevated the level of urinary 8-OHdG.

1747 QUANTIFICATION OF 3-NITROBENZANTHRONE DNA ADDUCTS USING ON-LINE SAMPLE PREPARATION AND HPLC-ELECTROSPRAY TANDEM MASS SPECTROMETRY.

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Sponsor: K. Delclos.

The aromatic nitroketone 3-nitrobenzanthrone (3-NBA) is an extremely potent mutagen and a suspected human carcinogen detected in diesel exhaust and in airborne particulate matter. A large body of evidence demonstrates that 3-NBA is a genotoxin, leading to the formation of covalent DNA adducts with purine bases via reduction of the nitro group to the hydroxylamine (*N*-OH-3-ABA). The predominant adducts formed *in vivo* upon exposure to 3-NBA or *N*-OH-3-ABA have previously been characterized as 2-(2'-deoxyguanosin-N^2-yl)-3-aminobenzanthrone (dG-N2-3-ABA) and *N*-(2'-deoxyguanosine-8-yl)-3-aminobenzanthrone (dG-C8-N-3-ABA). The detection and quantification of these covalent 3-NBA-DNA adducts has solely relied on ^{32}P-postlabeling. In order to expand the range of available techniques for the detection and quantification of these adducts we have developed a novel methodology based upon online sample preparation coupled with HPLC and electrospray tandem mass spectrometry with isotopic dilution of internal standards.
The preliminary results of the analysis of DNA samples modified *in vitro* with *N*-acetoxy-3-aminobenzanthrone and of DNA samples isolated from rodents treated with 3-NBA indicate that this methodology presents excellent linearity, precision and reproducibility within a broad range of DNA modification levels and an estimated limit of detection of 1 adduct/10^8 nucleotides, making it appropriate for biomonitoring purposes.

1748 MULTI-ADDUCT ANALYSIS OF GLOBIN FROM RATS AFTER INHALATION EXPOSURE TO 1, 3-BUTADIENE.

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Butadiene (BD) is an industrial chemical used in the production of synthetic rubber which is also found in gasoline and combustion products. BD is a multi- site carcinogen in rodents, with mice being the most sensitive species. BD is metabolized to several epoxides among which 1,2:3,4-diepoxybutane (DEB) is the most mutagenic. We present herein two immunoaffinity–LC-MS/MS assays for analysis of the 2-hydroxy-3-butenyl-valine (HB-Val), a biomarker for the formation of 1,3-epoxy-3-butene (EB), and the cyclic adduct N,N-(2,3-dihydroxy-1,4-butadiyl)-valine (pyr-Val), a biomarker for DEB. In short, globin samples were trypsin-hydrolyzed, filtered on Centricon-3 and applied to immunoaffinity columns. After extensive washing, elution and final filtration, the samples were analyzed by nano-UPLC-MS/MS. For exact quantitation we synthesized the internal standard peptides and quantified them based on amino acid analysis using LC-MS/MS. The methods were applied to analyze globin samples from male rats exposed to 625 ppm BD for 10 days by inhalation. In male rats, the amounts of HB-Val (582.9<±>133.8 pmol/g, n=5) were 3.5 fold higher than that of pyr-Val (166.8<±>23.3 pmol/g, n=5) at the same exposure. This multi-adduct analysis of globin provides valuable insight into the internal formation of individual BD metabolites. This approach will permit quantitation of the formation of EB and DEB following multiple inhalation exposures to BD.

1749 NOVEL LC-MS/MS METHODS FOR THE QUANTIFICATION OF THE MAJOR URINARY METABOLITE OF PROSTAGLANDIN E2 (PGE-M) AND CREATININE IN HUMAN URINE.

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Considerable attention has been devoted to studying the role of PGE2 in inflammation and cancer. Analytical methods measure chemically stable products or degradants of PGE2 to assess the biosynthesis of this prostaglandin due to its short half life (about 30 seconds in the blood). PGE-M (9,15-dioxo-11α-hydroxy-2,3,4,5-tetranor-prostan-1,20-dioc acid) is excreted into the urine and is the major urinary metabolite of PGE2 and a useful biomarker of systemic PGE2 concentrations. A new, facile, and robust LC-MS/MS method has been developed to measure PGE-M in human urine that involves the acid-catalyzed dehydration of PGE-M and its deuterated (d6) analog, sample extraction using 96-well solid phase extraction plates, and measurement of dehydrated PGE-M. We report also a new method for quantification of creatinine by LC-MS/MS in order to normalize PGE-M levels to the amount of creatinine excreted into the urine. Responses for both analytes were linear over a 1000-fold range. The intra- and inter-day precision for PGE-M ranged from 1.6% to 7.3% and 2.4% to 9.9%, respectively; PGE-M intra-day accuracy ranged from 97.8% to 106.6%, and inter-day accuracy ranged from 103.9% to 107.7%. Recoveries of dehydrated PGE-M following solid phase extraction varied from 96.0% to 100.4%. The intra-day precision for creatinine ranged from 0.4% to 1.3%, and the inter-day precision varied from 0.5% to 2.2%. Creatinine intra- and inter-day accuracies ranged from 94.4% to 106.9% and 95.0% to 104.2%, respectively. These methods were used to study the effect of aspirin (two doses of 650 mg) on urinary PGE-M levels in healthy male volunteers. There was no significant difference between pre- and post-aspirin in urinary creatinine concentrations. However, a significant decrease in the concentration of urinary PGE-M was observed following aspirin administration (mean decrease 41%; $p<0.01$). The application of these methods should facilitate greatly the evaluation of inflammatory disease states and the effectiveness of therapy in humans.

1750 DEVELOPMENT OF A NEW SYSTEM FOR THE IDENTIFICATION AND CLASSIFICATION OF AMPHETAMINE-DERIVATIVES ABUSER BY LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY.

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Amphetamine (AM) and man-made amphetamine-derivatives, methamphetamine (MA) and N,N-dimethylamphetamine (DMA), have been known as CNS stimulants, and their abuse throughout the world has recently increased. Since it difficult to physically distinguish among AM, MA and DMA, analysts may not be aware of what abusers have administered. In this study, following the detection of specific metabolites of AM, MA, DMA as biomarkers in abuser urine, a rapid and sensitive method could be developed for the classification of AM-derivatives abuser. After the direct protein precipitation with organic solvent, the urine samples were determined by LC/Triple-quadruple system under multiple reaction monitoring mode. Simultaneous identification of p-hydroxyl AM, p-hydroxyl MA, p-hydroxyl DMA, AM, MA, DMA and DMA N-oxide in urine could be achieved using a 10 min chromatographic run. To confirm the newly developed method, 10 samples from suspected AM-derivatives abuser, with the presence of MA and/or DMA confirmed in their urine samples, were applied. Of these 10 samples, 6 samples were decided to be originated from MA abusers, 1 sample was decided to be originated from DMA abuser.

1751 METABOLOMIC ANALYSIS OF PLASMA AND URINE IN RATS TREATED WITH FENOFIBRATE AND PHENOBARBITAL.

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A metabolomic analysis was performed to study the effects of fenofibrate and phenobarbital, two known rodent carcinogens, in rats. Plasma and 24-hour urine samples from rats treated with fenofibrate (300 mg/kg/day), phenobarbital (50 mg/kg/day), and vehicle control were collected at day 2 and day 14. Using a non-biased global profiling technology based on LC-MS and GC-MS, 496 and 974 compounds were measured from plasma and urine, respectively. We observed significant alterations in biochemical profiles by both drugs at both time points. Fenofibrate was found to increase fatty acid β-oxidation and down regulate glycolysis, resulting in a shift in energy production homeostasis. Phenobarbital was shown to impact various biochemical pathways, including carbohydrate metabolism and ascorbic acid biosynthesis. A group of toxicology markers related to liver and renal function, oxidative stress, and cell apoptosis were regulated in similar patterns by both fenofibrate and phenobarbital. Some of the toxicology markers were detected at day 2 and became more significant at day 14. The results demonstrated that metabolomics is an effective technology for studying drug mechanism and toxicology effects.

1752 CARBON NANOTUBE-BASED ELECTROCHEMICAL SENSOR FOR CHOLINESTERASE ENZYME ACTIVITY: AN ORGANOPHOSPHORUS INSECTICIDE AND NERVE AGENT EXPOSURE MONITOR.

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Simple, rapid and sensitive analytical sensors are needed to evaluate exposures to organophosphorus insecticides and nerve agents utilizing readily obtainable biological fluids, like saliva. An electrochemical sensor coupled with a micro-flow injection system was developed to characterize cholinesterase (ChE) enzyme activity. The sensor is based on a carbon nanotube (CNT)-modified screen-printed carbon electrode (SPE) integrated into a flow cell. The electrocatalytic activity of the carbon nanotubes, enable the sensor to detect electroactive species that are produced from enzymatic reactions with high sensitivity. The electrochemical properties of ChE enzymatic products were studied using the sensor, and the operation parameters such as the applied potential and substrate concentration were optimized. ChE activity was further investigated using the CNT-based sensor with purified human acetylcholinesterase (AChE) and *in vitro* with saliva obtained from naïve rats. For purified AChE the calibration curve was linear over a range of concentrations (5 pM to 0.5 nM) and the detection limit was estimated to be ~2 pM. The dynamics of salivary ChE activity was also studied *in vitro*. Paraoxon (0.7 nM and 7 nM) was mixed with 10-fold diluted saliva (1:1 v/v) and incubated for different time periods (5 to 120 min) then 100 μL of the mixed solution was reacted with an equal volume of 5 mM acetylthiocholine (~10 min) and sequentially assayed with the sensor. A concentration-dependent inhibition of salivary ChE activity was observed. At 0.7 nM paraoxon, the sensor response indicated ~80% enzyme inhibition within 0.5 h; whereas, at 7 nM 100% inhibition was achieved. Future studies will evaluate the sensor performance following *in vivo* exposure of rats to ChE inhibiting pesticides. This new CNT-based electrochemical sensor represents a potential next generation sensor for noninvasive biomonitoring of exposure to OP insecticides and chemical nerve agents. (Supported by CDC/NIOSH grant R01 OH008173-02)

1753 QUANTUM-DOTS-BASED ELECTROCHEMICAL IMMUNOASSAY FOR THE CHLORPYRIFOS AND TRICHLOPYR METABOLITE TRICHLOROPYRIDINOL.

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Chlorpyfios and trichlopyr are two routinely utilized pesticides that share a common urinary metabolite, trichloropyridinol (TCPy), which can be used to biomonitor for exposure. To facilitate biomonitoring there is a need to develop simple, rapid and sensitive sensors to evaluate exposures utilizing readily obtainable biological fluids, like saliva. A quantum-dot (QD)-based electrochemical immunoassay was developed to detect TCPy. QD conjugated with TCP derivative, triclopyr, was used as a label in the competitive immunorecognition event. TCP and the QD-labeled triclopyr competitively bind with the limited TCP antibodies on the magnetic beads (TCP-Ab-MB). The captured QD labels on the magnetic beads were determined by highly sensitive stripping voltammetric measurement of the dissolved metallic component (cadmium) with a disposable-screen-printed electrode. The parameters (e.g., amount of triclopyr-QD, amount of TCP Ab-MB and immunoreaction time) that govern the sensitivity and reproducibility of the electrochemical immunoassay were optimized. The voltammetric response is inversely proportional to the TCP concentration in the sample solution and is linear over the range of 0.05 to 1 ng mL^{-1} TCP. The limit of detection is estimated to be 0.02 ng mL^{-1}, which is 10 times lower than that of commercial enzyme linked immunosorbance assay kit (0.25 ng mL^{-1}). The performance of the developed immunoassay system was successfully evaluated on rat saliva samples spiked with TCP. This technique could be readily used for detecting other environmental contaminants by developing specific antibodies against contaminants and is expected to open new opportunities for biological monitoring. This new QD-based electrochemical sensor represents a potential next generation sensor for noninvasive biomonitoring of exposure to commercially important insecticides. (Supported by CDC/NIOSH grant R01 OH008173-02)

1754 MOLECULAR DOSIMETRY OF THE VINYL CHLORIDE-INDUCED DNA ADDUCT, 7-OXOETHYLGUANINE.

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Vinyl Chloride (VC) is an industrial chemical and known animal and human carcinogen which acts by a genotoxic mechanism. In the 1970s, it was shown that high level exposure of VC caused angiosarcoma of the liver (ASL) in VC workers. Early reports suggested that exposure to VC may also be associated with brain tumors. Using exposure to $[^{13}C_2]$-VC, we demonstrated in adult rats that neither N^2,3-ethenoguanine (EG), nor 7-oxoethylguanine (7-OEG) was detectable in brains of rats exposed to 1100 ppm. We continue this research by developing new LC-MS/MS methods for 7-OEG and applying these methods to analyze tissues from both adult and weanling rats exposed to 1100 ppm $[^{13}C_2]$-VC for 5 days. The 7-OEG was derivatized with O-t-butylhydroxylamine to an oxime adduct. Isotopically-labeled 7-OEG was synthesized by the reaction of chloroethylene oxide with dG. Following HPLC clean-up, it was characterized by MS and NMR and quantified by fluoresence. The level of 7-OEG in liver DNA was 28±6 adducts/10^6 nucleotides for adult rats exposed to $[^{13}C_2]$-VC (1100 ppm, 6hrs/day, 5 days) and 127±33 adducts/10^6 nucleotides for weanling rats exposed to $[^{13}C_2]$-VC (1100 ppm, 6hrs/day, 5 days). The method gave a detection limit of 15±2 adducts /10^8nucleotides in 5 ug DNA. To determine the persistence of 7-OEG, we found 74±19 adducts/10^8 nucleotides for animals sacrificed 2 wks post exposure, 17±2 adducts/10^8 nucleotides for 4 wks post exposure, and no adducts were found for 8 wks post exposure for adults rats exposed to [^{13}C2]-VC (1100 ppm, 6hrs/day, 5 days). Additionally, we will explore the formation of 7-OEG in the brains of VC exposed weanling and adult rats.

1755 A NOVEL APPROACH FOR ANALYSIS OF N-TERMINAL VALINE ADDUCTS AS BIOMARKERS FOR EXPOSURE TO ALKYLATING COMPOUNDS.

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Protein adducts are well established biomarkers for alkylating agents and are used to study environmental or occupational exposures. Thus far, these adducts have been measured by modified Edman degradation, immunohistochemistry or by analysis of the tryptic fragments after immunoaffinity chromatography (IA). However, these highly specific and sensitive methods usually allow only quantitation of a single adduct. To overcome this limitation we present here the proof-of-concept for simultaneous analysis of multiple adducts. Rather than using antibodies specific to the adduct of interest, antibodies were raised against the C-terminus of the rat N-terminal tryptic peptide, and 'selection' immunoaffinity columns were prepared. This antibody has high specificity towards the rat peptide whether its N-terminal valine is alkylated or not. The recovery of diepoxybutane (DEB) derived N,N-(2,3-dihydroxy-1,4-butadiyl)-valine (pyr-Val) was ~ 93%. Analysis of peptide standards or globin treated in vitro with 1,3-epoxy-3-butene (EB), DEB or EB plus DEB demonstrate the suitability of the IA columns to enrich N-terminal peptides. However, since these IA columns also enrich normal N-terminal peptides, interference and poor recovery was observed for low level pyr-Val standard peptides (2 pmol) in presence of larger amounts (5mg) of globin. Subsequently, antibodies were raised against the unmodified N-terminal peptide and 'depletion' immunoaffinity columns were prepared. The recoveries of pyr-Val and the EB derived 2-hydroxy-3-butenyl-valine peptide standards in globin after trypsin hydrolysis, de-

pletion and selection IA chromatography were 52% and 50%, respectively. In conclusion, a new approach for selective enrichment of adducted N-terminal peptides has been proven to be suitable for analysis of in vitro treated rat globin. It is hypothesized that this approach will allow simultaneous bio-monitoring of multiple alkylating agents of interest to environmental health researchers.

1756 CONSTRUCTION OF TISSUE SPECIFIC HIGHLY-EXPRESSING GENES DATABASE FOR BIOMARKER HUNTING.

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Databases composed of tissue specific highly-expressing genes in various tissues or organs are extremely useful tools to identify the distribution of drug-target molecules and to evaluate drug-induced toxicity in target tissues and/or organs. In particular, the databases are invaluable for searching for biomarkers for leaking enzymes from damaged tissues, for the evaluation of the differentiation of ES cells and for the identification of differentiated tissues. In this study, tissue specific highly-expressing genes in the mouse were identified using a gene expression database. A simple extraction method was applied to obtain tissue specific highly-expressing genes because the statistical method for outlier detection did not work suitably due to the diversity of the gene-expression values.
Seventy-two gene expression datasets obtained with the Affymetrix Mouse Genome 430 2.0 Array were used. The datasets consist of 20 tissues or organs which were excised from male C57BL/6 mice and were as follows: adipose tissue, bladder, blood, bone, brain, breast, colon, esophagus, heart, left kidney, right kidney, liver, lung, pancreas, prostate, skeletal muscle, spleen, stomach, testis and thymus. The tissue-specific highly-expressing genes were defined as those individual gene-expressing values in the target tissue that were at least two-fold greater than the highest value in the other tissues. Comparing the individual gene-expressing values of all the tissues or organs, we determined from 2 to 4669 tissue-specific highly-expressing genes, probesets, in every tissue except for the left kidney, right kidney, skeletal muscle and prostate. In addition, latent tissue-specific genes were detected by a combination of data from tissues which have no tissue-specific genes alone.
The extraction method for tissue specific-expressing genes described in this study could be applied to any type of gene expression database. The tissue specific highly-expressing genes can give helpful and fundamental information in the research and development of drugs.

1757 QUANTITATION OF SELECTED CONSTITUENTS IN UNDILUTED MAINSTREAM CIGARETTE SMOKE GENERATED BY DIRECT PUMP EXPOSURE SYSTEMS USING UNFILTERED 2R4FS.

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Small-scale direct pump (SSDP) cigarette smoke exposure systems (SES) such as the system manufactured by Washington University in St. Louis (WUStL) are widely used to generate undiluted mainstream smoke (MS) to expose rodents in studying cigarette smoke-induced diseases. In this study, MS generated by WUStL SES using 2R4F reference cigarettes (filter removed) was characterized for selected smoke constituents. Intra-system port and inter-system smoke concentration (conc.) variability were measured over time. System operation consisted of a ~20 mL puff volume per port for ~30 sec per puff. Average ± SD puff counts were 9.0 ± 1.1 puffs per cigarette. Smoke nicotine, wet total particulate matter (WTPM), and carbon monoxide (CO) were measured using 4 cigarettes per sample from 4 SES at selected times over ~6 months. Samples were collected from the end of silastic transfer tubing normally connected to animal containment boxes. WTPM and nicotine samples were collected using Cambridge filters coupled with sorbent tubes. Filters and tubes were analyzed by GC/FID for total nicotine. CO was collected using Tedlar® gas bags and analyzed by GC/TCD. Average ± SD conc. of MS were 4,733 ± 1226 µg nicotine/L, 17,415 ± 4766 µg WTPM/L, and 13,788 ± 3157 ppm CO, with the average ratios to WTPM of 274 ± 17.5 nicotine/WTPM (µg/L/mg/L) and 0.9 ± 0.3 CO/WTPM (mg/L/mg/L). Compared to automated SES, average MS conc. of the WUStL system for nicotine, WTPM and CO were ~110, 25, and 20-times higher, respectively, than values measured in 600 µg WTPM/L diluted MS generated by a 30-port Condor SES using filtered 2R4Fs. Therefore, this kind of SSDP cigarette SES using unfiltered cigarettes can generate extremely high conc. of smoke constituents over a relatively short exposure duration. Pre-conditioning of the SES was necessary to produce relatively consistent smoke output. (Funded by Battelle Biology and Health Science Initiative).

1758 COMPARATIVE PERFORMANCE OF NOVEL MARKERS OF NEPHROTOXICITY IN THE RAT.

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The HESI renal biomarker consortium evaluated the performance of 4 novel urinary biomarkers (BMs); α and µ-GST, clusterin and renal papillary antigen-1(RPA-1). Elisa assays (Biotrin) were analytically validated within and between laboratories (Harpur et al., 2007, Supp. to Toxicologist, 96:1). Biological data were generated using 3 renal toxicants, cisplatin, gentamicin and N-phenyl anthranilic acid, administered to male rats of two strains, Han Wistar and Sprague Dawley. The resulting renal injury, which varied across studies in incidence, severity and nephronal segment, was recorded according to a pre-determined lexicon of diagnostic terms. For each compound the localization of the injury was determined by immunohistochemistry. The diagnostic performance of the novel BMs was assessed using histopathology (HP) as the standard and compared with conventional markers of renal function (BUN and serum creatinine (Scr)) and conventional urinary BMs of tubular injury (total protein and NAG). The relative sensitivity and specificity with which each BM reported nine HP diagnoses was statistically assessed using Receiver Operating Characteristic (ROC) curve analysis. For each BM and HP diagnosis, the area under the ROC curve was calculated as an overall measure of diagnostic performance. Both the conventional urinary BMs (protein and NAG) as well as the novel BMs out-performed BUN and Scr. For some HP diagnoses, the novel BMs out-performed urinary protein and NAG, e.g. α-GST for PT injury. RPA-1 provides for the first time a urinary marker for CD injury enabling non-invasive monitoring of renal papillary injury. Overall, the performance of these novel urinary BMs was superior to that of the traditional renal BMs and provided the additional benefit of information on localization of the segment of injury.

1759 UPLC-ESI-MS/MS QUANTITATION OF CISPLATIN GUANINE INTRASTRAND CROSS LINKS IN OVARIAN CARCINOMA CELL LINES.

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Platinum chemotherapeutic agents have been successfully used in the treatment of various cancers for over 30 years. However, it is not known if detoxication or DNA repair of specific adducts are responsible for varied responses to treatment. Previous methods for measuring platinum adducts have lacked sensitivity and specificity. The most recently published monoclonal antibody immunoassay shows cross reactivity for the guanine-guanine and adenine-guanine intrastrand cross links. Atomic absorption and ICP-MS both measure total platinum, which does not provide information about individual types of adducts. Finally, previous LC-MS methods did not use an internal standard, making these assays semi-quantitative. Biochemical studies have shown the most prevalent DNA modification is formed between two adjacent guanines at the N7 position, producing the intrastrand cross link between cisplatin and guanine(CP-d(GpG)). We developed a method for analysis of CP-d(GpG) spiked DNA after SPE clean up with selected reaction monitoring (m/z 824→496) that resulted in a limit of detection of 1 CP-d(GpG) adduct per 10^7 nucleotides using an internal standard. Cisplatin sensitive (A2780) and acquired resistance (CP70) ovarian carcinoma cell lines were treated with cisplatin (0, 12.5, 25, 50, 100, 250µM) for 1 hr followed by DNA isolation, sample clean up and UPLC-ESI-MS/MS quantitation. A linear dose response was observed with approximately twice the adducts formed at each dose in the more sensitive A2780 cells. Literature has shown as much as a 13-fold increase in GSH levels for the CP70 vs. the A2780 cells. Therefore, the observed adduct difference is likely to be attributed to greater detoxication. Previous studies using these cell lines required the use of large doses (250-750µM) to measure adducts via atomic absorption. Our method allows quantitation with only 10 µg DNA at doses at or below the IC50. The sensitivity, specificity, and minimal DNA requirement of this new method will allow molecular dosimetry studies following cisplatin treatment.

1760 MATRIX METALLOPROTEINASE-9 (MMP-9)-MEDIATED EXTRACELLULAR MATRIX REMODELING CAN BE DETECTED USING GRATING COUPLED SURFACE PLASMON RESONANCE IMAGING.

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Matrix metalloproteinase-9 (MMP-9) is a gelatinase secreted by monocytes and macrophages. This enzyme is upregulated during tissue remodeling, and in the course of rheumatoid arthritis (RA) and certain types of cancer. We have recently

shown that proteolytic digestion of type IV collagen by MMP-9 can be detected using grating-coupled surface plasmon resonance imaging (GCSPRI). GCSPRI is a highly sensitive real-time, label-free technique capable of detecting mass-dependent changes on a molecular scale. Surface plasmons are generated at a metal-dielectric interface when light of a specific wavelength couples into electrons at the gold surface at the specific angle of incidence. Small changes in the incident angle indicate a mass-dependent interaction between analyte in solution and immobilized protein on a gold chip. Traditionally GCSPRI has been used to analyze increasing mass at the sensor chip surface (as a consequence, for example, of antibody-antigen interactions) but we have recently shown that enzymatic activity of immobilized type IV collagen by both collagenase from Clostridium histolyticum and human MMP-9 can be detected by GCSPRI. We have also observed digestion of the same substrate by enzymes secreted from activated U937 macrophages. These studies indicate that measurements of matrix metalloproteinase specific activity can be a component of the biological signature for specific disease processes. (Supported by NIH award #ES07408)

1761 A TIME AND DOSE RESPONSE METABONOMICS STUDY OF D-SERINE TOXICITY IN RATS.

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Toxicity of D-serine, a model nephrotoxin, was evaluated by NMR-based metabonomics and multivariant analyses. Rats were given a single dose of either saline (vehicle) or D-serine at 5, 20, 50, 200, or 500 mg/kg (ip). Urine and plasma were collected predose and postdose for 4 days; kidneys were removed at 96 h for histopathology. Blood chemistry showed significant effects at doses ≥200 mg/kg ($p<0.05$). At 24 h postdose plasma total bilirubin (TBIL) decreased 40%, while urea nitrogen (BUN) and creatinine (CREA) increased 5-fold and 3-fold, respectively. By 96 h, the 200 mg/kg group showed recovery, but CREA and BUN remained significantly elevated for the 500 mg/kg dose. Histology showed no kidney abnormalities at ≤50 mg/kg; however, moderate effects were observed on tubular eosinophilic fluid and nephropathy at ≥200 mg/kg. Urine was analyzed by 1H-NMR (14.1 T) and principal component analysis (PCA) of "binned" spectra. PCA scores plots (PC1 vs PC2) for each treatment dose vs vehicle control across all time points revealed a clear separation at doses ≥200 mg/kg. The onset of toxicity and maximum excursion in PC-space occurred at 1 day postdose, with recovery towards predose coordinates at days 2-4. Salient features were identified by linear discriminant analysis (LDA) and bin-by-bin t-tests with appropriate corrections for multiple testing and vehicle effects. Dose-dependent urinary metabolite changes were observed. At 1 day post-500 mg/kg, 65 highly significant bins were identified by t-tests ($p<0.0001$) and LDA (top 20% coefficients) methods combined. Spectral assignments showed that D-serine caused a significant increase in urinary valine, alanine, glucose, betaine, and lysine, and a decrease in 3-phenylpropionate, N-methylnicotinate, N-methylnicotinamide, methylamine, and trans-aconitate. Further refinement of our statistical tools is enabling the identification of urinary biomarkers for chemical-induced toxicity. Funded by AFRL, Henry M Jackson Foundation, Contract No. 132633.

1762 PLASMA VON WILLEBRAND FACTOR PROPEPTIDE (VWFPP) IS A POTENTIAL PREDICTIVE DRUG-INDUCED VASCULAR INJURY (DIVI) BIOMARKER.

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Pathological findings of DIVI in preclinical toxicology studies complicate the drug development process due to a lack of predictive biochemical markers. One approach used by the Predictive Safety Testing Consortium (PSTC) vascular working group (VWG) to identify potential biomarkers was to investigate proteins released from the primary targets of DIVI, endothelial (EC) and smooth muscle cells. One protein of particular interest was VWFpp because it is clinically used as a biomarker of EC perturbation. However, the human VWFpp ELISA did not cross-react with the dog. The goal of this research was to develop a canine specific VWFpp ELISA, qualify it as an EC perturbation biomarker and evaluate it as a potential DIVI biomarker. Four canine specific VWFpp clones were produced with two selected as the capture and detection antibodies. The ELISA passed a standard validation protocol. DDAVP and endotoxin, used to evaluate VWFpp as an EC perturbation biomarker in humans, were used to qualify canine VWFpp as an EC perturbation biomarker. Similar to the finding in human, DDAVP induced a rapid but short duration increase in canine VWFpp (9 fold increase at 1 hour; returned to baseline by 3 hours) and endotoxin induced a rapid but prolonged increase (70 fold increase at 1 hour; 2 fold increase still present at 24 hours). Several compounds were used to evaluate VWFpp as a potential DIVI biomarker. Canine VWFpp was increased only when blood was collected within 6 hours of dosing and a dose dependent increase was observed at 1 hour post-dose. Optimization of sample collection may be necessary as high VWFpp was detected in a few pre-dose samples. Based on these findings canine VWFpp is a potential predictive DIVI biomarker that should undergo further qualification.

1763 CYTOKINE mRNA PROFILING DIRECTLY FROM *EX VIVO* STIMULATED MOUSE WHOLE BLOOD.

O. N. Diallo, A. Keightley and L. LeSauteur. *Toxicology, Charles River Labs, Preclinical Services Montréal Inc., Senneville, QC, Canada.* Sponsor: C. Banks.

Gene expression profiling is a valuable biomarker endpoint in toxicology. We developed and validated a QuantiGene® Plex assay for direct multiplex quantitation of cytokine mRNA expression in a small volume of ex vivo stimulated mouse whole blood.
Peripheral whole blood samples were collected from CD-1 mice and stimulated with 1 μg/mL of E. coli lipopolysaccharide, phorbol myristate acetate and ionomycin. RNA was released from the cells by lysis and target RNAs were captured in the whole blood lysate by overnight hybridization with Luminex xMAP® capture bead arrays and probe sets specific for IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12 (p40), IL-17, TNF-α, IFN-γ and GM-CSF, as well as for housekeeping genes GAPDH, β-actin, and PPIB. The overnight mixture was then subjected to signal amplification with branched DNA amplifier and biotinylated label probe. Incubation with streptavidin-conjugated R-phycoerythrin provided the signal detection and the resulting fluorescence signal was read by the Bio-Plex Suspension Array System.

The signals for each mRNA target in the cytokine panel were normalized to the fluorescence signals for the housekeeping genes. PPIB mRNA expression was more stable than either GAPDH or β-actin, displaying the lowest level of inter-sample variability. Results for the treated blood samples were expressed as fold induction compared to untreated control blood. IL-1β, IL-6, IL-10 and TNF-α were substantially upregulated with a peak induction at 2 hours (17-, 4-, 28-, and 18-fold minimum induction, respectively). The mean intra-assay precision was below 10% for the housekeeping genes and below 25% for all genes. The inter-assay precision for the cytokines was more variable due to inter-individual differences.

This method provided robust, precise and specific quantitation of cytokine gene expression directly from mouse whole blood samples without the need for RNA purification, reverse transcription or target amplification. This method is suitable for accelerated toxicity evaluation of potential drug candidate effects on the immune system

1764 AN ELISA MICROARRAY PLATFORM FOR USE IN HIGH-THROUGHPUT SCREENS OF BIOLOGICAL FLUIDS.

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The sandwich enzyme-linked immunosorbent assay (ELISA) microarray can quantitatively measure rare proteins in complex biological fluids. The advantages of this platform are high-throughput potential, assay sensitivity and stringency, small sample volumes, and the similarity to the standard ELISA test. Major concerns include efficient processing of the data, controlling for variability across chips, and retaining assay specificity when multiplexing. We have developed a specialized program (ProMAT) for rapid analysis of ELISA microarray data; and have integrated this program in a MIAME-compliant LIMS database, the BioArray Software Environment (BASE). We have also developed an internal calibrant that improves assay reproducibility and sensitivity. In designing each chip, we employ a systematic approach for determining the amount of assay interference and noise contributed by individual components of the multiplexed assay system. We find that non-specific reagent cross-reactivity problems are relatively rare. Unexpectedly, a significant problem is the presence of contaminant antigens in "purified antigens" or in blocking solutions. We tested the validated ELISA microarray chip using paired serum samples that were collected from four women at a 6-month interval. This analysis demonstrated that protein levels typically vary much more between individuals then within an individual over time. Overall, this research demonstrates the importance of a stringent screening protocol and the value of optimizing the antibody and antigen concentration when designing chips for ELISA microarrays. This work funded by NIH grants CA117378 and EB006177.

1765 EFFECTS OF CIGARETTE SMOKING IN PREGNANCY ON THE PLACENTA VASCULOSYNCYTIAL MEMBRANE THICKNESS.

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Objective: To determine the effect of cigarette smoking(CS) during pregnancy on placental vasculosyncytial membrane thickness(VSM).
Methodology: A detailed history of smoking was obtained in all participants. Maternal serum cotinine, a reliable marker of CS exposure(≥25 ng/ml), was measured in 334 pregnant woman at delivery. Fifteen women with cotinine levels ≥25 ng/ml and self reported history of CS during pregnancy as well as 15 women with cotinine levels ≤1ng/ml that have never smoked(NS) were studied. Gestational age(GA), birth weight(BW), placental weight, and fetal placental ratio(FPR) were recorded. Furthermore, VSM thickness was measured by two pathologists blinded to smoking status in H&E stained placenta sections(3 μm) examined at 100X oil immersion. VSM of 40 terminal villi located in the middle section of the slide were measured for each placenta. The narrowest spot at the trophoblast barrier was selected consistently to measure the VSM thickness. Results were expressed as mean and standard deviation as well as medians and interquartile ranges. T-test and Mann-Whitney test were used to compare differences between groups (α=0.05). Log transformations were used as required.
Results: No statistical differences were documented in GA comparing CS vs. NS [39(38-39) vs. 39(38-40),p=0.643]. BW was significantly lower in CS compared with NS [3315 ± 690 vs. 3776 ± 439)gr.,p=0.04]. Autolytic changes were noted in four of the participant's placental slides and therefore were excluded for VSM analysis [CS=1, NS=3]. Increased VSM thickness was documented in CS compared with NS [0.34 ± 0.09 vs. 0.27 ± 0.07 log μm,p=0.038]. Although no statistical differences were found in placental weight or FPR between groups, there was a trend towards larger placentas in CS compared with NS.
Conclusions: CS exposure during pregnancy is associated with increased VSM thickness. We propose that CS toxicity to the syncytial trophoblasts may cause delayed villous maturation resulting in increased VSM thickness.

1766 THE CONTRIBUTION OF DENTAL AMALGAM TO URINARY MERCURY EXCRETION IN CHILDREN.

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Urinary mercury concentrations are widely utilized as a measure of mercury exposure from dental amalgam fillings. No reports have described the relationship of these measures in a longitudinal context in children. In this study we evaluated urinary mercury in children aged 8-18 in relation to number of amalgam surfaces and time since placement over a 7-year course of amalgam treatment. 507 children, ages 8-10 at baseline, participated in a clinical trial to evaluate the neurobehavioral effects of dental amalgam in children. Subjects were randomized to either dental amalgam or resin composite treatments. Urinary mercury and creatinine concentrations were measured at baseline and annually on all participants. Treatment groups were comparable in baseline urinary mercury concentration (~1.5 μg/L). Mean urinary mercury concentrations in the amalgam group increased to a peak of ~3.2 μg/L at year 2, then declined to baseline levels by year 7 of follow-up. There was a strong positive association between urinary mercury and both number of amalgam surfaces and time since placement. No significant changes in urinary mercury excretion were observed among subjects with composite resin treatment. The findings demonstrate that urinary mercury concentrations are highly correlated with both number of amalgam fillings and time since placement in children. Notably, girls excreted significantly higher concentrations of mercury in the urine than boys with comparable treatment, suggesting possible gender-related differences in mercury handling and susceptibility to mercury toxicity. Received both UW and UL IRB approvals. Supported by NIH U01DE11894.

1767 DIFFERENCES IN ARSENIC METABOLISM BETWEEN MEXICAN BOYS AND GIRLS.

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Arsenic is an element that has been associated with cancer in adults and decreased neurodevelopment in children. Several studies have suggested that women have a greater capacity than men to metabolize arsenic. However, gender differences in arsenic metabolism in children have not yet been demonstrated. Our aim was to evaluate possible differences in arsenic metabolism between boys and girls, using methylation ratios of As metabolites in urine as a biomarker. We conducted a cross-sectional study on 591 children ages 6-8 years old living in Torreón, Mexico, an area highly contaminated with heavy metals such as arsenic and lead. Urinary levels of arsenic and its metabolites (MMA and DMA) were determined by hydride generation atomic absorption spectrophotometry. The sum of all arsenic species (AsT) was categorized by ≤35 μg/L, 35.01-50 μg/L, and >50μg/L. We compared the relationship between AsT and the ratios of first methylation (MMA/Asi), second (DMA/MMA), and total methylation (DMA/Asi) between boys and girls using multivariate linear regression models. Increases in AsT above 35.01 μg/L appear to limit As methylation. The magnitude of the decrease in methylation of Asi to MMA (Ratio MMA/Asi) was significantly lower in girls than boys (p<0.02), while a decrease in the methylation to DMA (DMA/MMA) attributable to increased levels of arsenic was not significantly different between genders (p<0.60). There was no significant effect of lead on arsenic metabolism in either boys or girls. Our results suggest differences between boys and girls in the methylation of inorganic arsenic to MMA. Further studies are needed to determine the nature of these gender differences.

1768 MASS SPECTROSCOPY, AND NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY ANALYSES OF OXYLIPIDS, LIPIDS, AND AMINO ACIDS IN PLASMA OF NON-HUMAN PRIMATES EXPOSED TO ENVIRONMENTAL TOBACCO SMOKE DURING PERINATAL DEVELOPMENT.

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Metabolomics can serve as a highly sensitive measure to detect subtle alterations in physiologic and biochemical functions caused by exposure to environmental factors, such as Environmental Tobacco Smoke (ETS), during gestation and early childhood which may result in disease in later life. The measurement of systemic metabolites and the regulators of cellular function may serve to identify early indicators of disease. To better understand potential implications of ETS exposure to nonhuman primates, rhesus macaque infants were to exposure to ETS during gestation and early postnatal life at a concentration of 1mg/m3 total suspended particulates for 6 hours per day, 5 days per week. Two treatment groups were involved: 1) ETS from gestation day 40 to 3 months, 2) sham-control / filtered air. Plasma was examined using nuclear magnetic resonance spectroscopy (NMR), fourier transform ion cyclotron mass spectrometry (FI-ICR-MS), and liquid chromatography/mass spectrometry/mass spectrometry (LC-MS/MS). FT-MS data focused on masses in the 700- 1000 m/z region where phospholipids are observed. LC/MC/MS examined oxlipin inflammatory mediator profiles. NMR analyses looked at amino acid changes. Mass-aligned FT-MS data of plasma samples showed separation between the control and the smoke exposed groups for month 2. Separation between the control and smoke exposed groups were seen in month 1 and 3 using PLS-DA. Results of the oxlipin profiling showed a significant different in several eoscoinoids at the three month time point including a decrease in DHOME, and PGF2 along with an increase in 5,6 DHET. . NMR analyses also showed significant changes at the three month time point. This data supports the use of multiple analytically approaches to metabolite profiling for the most comprehensive view of systemic changes in environmental pollutant exposures

1769 SUPPRESSION OF PPARγ ACTIVITY CAUSES INCREASED PROLIFERATION AND DECREASED NEUTROPHIL ACCUMULATION IN THE LUNGS OF NEWBORN MICE EXPOSED TO >95% O2.

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Premature infants often require hyperoxic therapy for survival and hyperoxia is associated with inflammatory responses and alterations in lung development. PPARγ regulates pathways involved in both inflammation and proliferation. Previous studies indicated hyperoxia-dependent decreases in PPARγ expression in newborn C3H/HeN mouse pups, We tested the hypothesis that administration of a PPARγ antagonist would exacerbate hyperoxia-induced lung inflammation and further inhibit lung development. C3H/HeN mouse pups were housed in room air (RA) or >95% O2, were dosed daily with 10 μg/kg GW9662, a PPARg antagonist, or vehicle ip, and were sacrificed at 1, 3, 7 or 14 days of exposure. Pups exposed to RA + GW9662 had a greater number of alveoli by day 14 than did those pups exposed to RA + vehicle or pups exposed >95% O2 or >95% O2 + GW9662 (89±3, 61 ± 4,

29 ± 5, 28 ± 2, n=3), suggesting that PPARγ suppression caused a shift toward proliferation and away from differentiation. At days 3, 7, and 14 pups exposed to >95% O2 + GW9662 had fewer neutrophils per high power field (12.8 ± 3.8, day14) than did pups exposed to >95% O2(41.1 ± 3.8, day 14) alone but greater levels than RA (4.1±0.3, day 14) or RA + GW9662 (3.5 ± 0.4, day 14). 5-lipoxygenase provides substrate for leukotriene production during inflammation. Administration of GW9662 caused a diminution of the oxygen-induced increases in 5-lipoxygenase by day 7 (1.9 ± 0.4, O2 and 1.1 ± 0.2, O2+GW, relative density assessed by Western blot) indicating that decreases in PPARγ signaling may be related to a reduction of 5-LO protein expression. In summary, suppression of PPARg activity by GW9662 caused increased proliferation and decreased neutrophil counts and 5-LO expression. These data support the finding that PPARγ expression is anti-proliferative but also may indicate alternative pathways affected by PPARγ supression (Supported by the NIH, HL068948).

1770 LOW-DOSE EXPOSURE OF NEONATAL RATS TO THE ANTIMALARIAL, β-ARTEETHER (βAE), RESULTS IN BRAINSTEM PATHOLOGY WITHOUT OBVIOUS BEHAVIORAL CHANGES.

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Up to 500 million people contract malaria each year. At least 2.7 million people die annually from the illness, 90% of whom are pregnant women and children. With the presence of drug-resistant *P.falciparum*, combinatorial treatment regimens to include highly efficacious Artemisinin derivatives are recommended by W.H.O. Although very effective against resistant malarial strains, βAE in adult clinical studies is shown to be associated with ototoxicity, tremor and other autonomic and motor impairments. Similar behavioral symptoms and in addition brainstem lesions are observed in acutely βAE treated adult laboratory species (mice, rats, dogs, and monkeys). To date there have been few long term studies conducted with laboratory animals and none initiated in neonatal or juvenile animals. In this study, rat pups (7-days old) were treated with 1 - 90 mpk βAE (intramuscularly in sesame oil) up to 8 treatment cycles (1 cycle = 7 days treatment + 7 days no treatment). Neonates were tested for sensorimotor changes, and when adult, were tested using the Functional Observational Battery (FOB), motor activity, and the 8-arm radial maze (8ARM) to assess the ability to learn and remember the spatial location of bait. Pups receiving 60 and 90 mpk died within a week of treatment initiation. Rats receiving 10 mpk showed slight behavioral changes in the FOB and 8ARM after 7 cycles, whereas rats receiving 1 or 5 mpk showed no behavioral changes. Histopathology showed brainstem lesions after 5 cycles of 1 mpk (the lowest dose level tested), including cytoplasmic eosinophilic inclusions, nuclear pyknosis and karyolysis, and neuronal satellitosis of the vestibular and olivary nuclei whereas early high-dose deaths had no brainstem changes. This study shows that repeated treatment of clinically relevant doses of βAE causes brainstem damage in rodents and suggests βAE may cause subclinical brainstem damage in children and that repeated dosing may be required to elicit neurological damage. Work supported by NIAID Contract N01-AI-05417.

1771 EXPRESSION PROFILE OF THE MAJOR CYTOCHROMES P450 IN NEONATAL CD-IGS RATS.

L. Nguyen, J. Nash, S. Gagnon, L. Pinsonneault and K. Robinson. *Toxicology, Charles River Labs, Preclinical Services Montréal Inc., Senneville, QC, Canada.* Sponsor: C. Banks.

Juvenile and reproductive toxicology studies required for preclinical drug development of drug candidates are often conducted with CD-IGS rats. Several studies on the age-related expression of CYP450s in rats were reported in the literature, but none focused on the activity quantification for the first 4 weeks post partum. In this study, we determined the early expression of the major inducible CYP450s in neonatal CD-IGS rats (Crl:CD(SD)) between 4 and 30 days post partum using probe substrates, as this period is crucial for the detoxification of endobiotics and xenobiotics. Neonatal male and female rats (total of 120) were euthanized at 4, 7, 10, 14, 17, 21, 24 and 28 days post partum. Four to eight animals per timepoint per gender were used for the preparation of liver microsomes. The total P450 and b5 content was measured spectrophotometrically, while the activities of CYP1A1/2, CYP2B1/2, CYP2E1 and CYP3A1/2 were determined with EROD, PROD, chlorzoxazone and testosterone probe substrates, respectively. A slight increase in the total P450 (~25%) and b5 (~50%) content was observed from day 4 to 28 post partum. The specific activity of CYP1A1/2 activity increased 12-fold (35 to 420 pmol/mg/min), with peak activity at 24 days, while CYP2B1/2 activity remained at a similar level (~ 36 pmol/mg/min) from 4 to 28 days. CYP2E1 exhibited a 2.6-fold increase of activity (~1.3 to 3.4 nmol/mg/min) while a modest but steady increase of CYP3A1/2 activity was noted between 4 and 24 days (1.1 to 1.3 nmol/mg/min). These changes appeared to be consistent in both male and female rats, with no significant difference between genders. Our laboratory historical data indicated that the 9-week old rats showed significant suppression of CYP1A1/2 and CYP2B1/2 activities, while the CYP3A1/2 subfamily exhibited a 4-5 fold increase. Thus, the CYP450 expression in neonatal CD rats was age-dependent for the major P450s, with peak activity for CYP1A1/2 and CYP2E1 at 24 days post partum, and a steady increase of CYP3A1/2 activity to at least 9 weeks post partum.

1772 RAT AND DOG CLINICAL PATHOLOGY HISTORICAL CONTROL DATA FOR NEONATAL AND JUVENILE TOXICOLOGY STUDIES.

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The use of pharmaceutical and biotechnology products to treat pediatric patients requires that the conduct of neonatal and juvenile toxicology studies be considered before clinical trials are conducted. Rats and dogs are often selected as models for these non-clinical studies (FDA/EMEA). Clinical pathology data has shown to be useful in assessing toxicity in these neonatal and juvenile toxicity studies. Studies have been conducted in CD-IGS rats and Beagle dogs utilizing a variety of dose routes and including clinical pathology tests. Sampling techniques varied according to the age of the animals. Fasting prior to sample collection typically cannot be implemented prior to or soon after weaning. Small sample volumes may require pooling of samples within litters and assaying of limited parameters at younger ages. Compilation of control data by age for each species showed changes across time that were similar in both species. These changes included decreasing reticulocyte counts and increasing erythrocyte counts and hemoglobin values with age. Changes in clinical chemistry parameters related to age for rats included reductions in bilirubin and alkaline phosphatase and increases in serum proteins. In dogs, age related decreases in triglycerides and cholesterol and increases in asparate aminotransferase and alanine aminotransferase for were commonly noted. Interpretation of the clinical pathology data of neonatal and juvenile toxicology studies can be aided by the availability of historical control data from comparable ages of animals.

1773 INHALATION JUVENILE RAT STUDIES CAN BE PERFORMED AS EARLY AS AT DAY 4 POST PARTUM.

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In order to test the feasibility of nose-only inhalation exposure in neonatal rodents, an inhalation study with Verapamil hydrochloride (CAS No. 152-11-4) as a model compound was conducted in neonatal rats aged day 4-21 post partum (pp). End points for the evaluation were adverse clinical signs, the body weight gain during development, substance serum concentrations for kinetic analysis and possible cross-contamination between dams and littermates.

Standardized litters were assigned to the following groups: cage control, vehicle control, Verapamil treatment, Verapamil kinetic analysis and Verapamil cross-contamination analysis. For the cross-contamination kinetic approach, 3 litters were divided between vehicle control and substance exposure during treatment but raised together. Treatment was carried out for 2 hours daily from day 4 until day 21 pp. The exposure concentration was set at 50 mg/m3 Verapamil with a substance mass median aerodynamic diameter of 0.5 µm.

The results gave no indication of adverse clinical signs in neonates due to maternal deprivation for 2 hours. No increased mortality was observed. The cumulative body weight gain in the treatment groups vs. cage control was comparable. An efficient serum recovery of Verapamil as early as day 4 pp was achieved with consistent serum levels of 214, 274 and 276 ng/ml at day 4, 10 and 21 pp respectively. Cross-contamination analysis gave no evidence of a carry-over via lactation and maternal behaviour or between littermates.

Neonatal rats aged 4-21 days depict human pulmonal development stages between preterm newborns and children of about 6-8 years of age. The practicability of inhalation treatment in juvenile rats as young as day 4 pp permits new insights into human neonatal risk assessment and therefore this animal model is suitable for regulatory studies.

1774 ATTENUATION OF HYPEROXIC LUNG INJURY BY THE CYTOCHROME P450 (CYP)1A INDUCER β-NAPHTHOFLAVONE (BNF) IN NEONATAL WILD TYPE AND CYP1A2-NULL MICE.

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Supplemental oxygen administration is frequently encountered in the treatment of infants having pulmonary insufficiency. However, prolonged hyperoxia causes lung damage, and contributes to the development of bronchopulmonary dysplasia

(BPD) in infants. In this investigation, we tested the hypothesis that pre-treatment of newborn mice wild type (WT) (C57BL/6J) or CYP1A2 knockout, followed by exposure to hyperoxia, would lead to attenuation of lung injury by mechanisms involving CYP1A-mediated detoxification of F2-isoprostanes. Newborn WT or CYP1A2-null mice (3 day-old), delivered from timed pregnant mothers, were pretreated with the vehicle corn oil (CO) or BNF (80 mg/kg), i.p., once daily for 4 days, and animals were maintained in room air or exposed to hyperoxia (> than 95% O2) for 1-7 days. After 7 days of hyperoxia, the lungs of CO-treated WT and 1A2-null showed pulmonary edema and inflammatory cells, with 1A2-null animals showing greater injury. On the other hand, BNF-pretreated neonatal mice showed decreased susceptibility to hyperoxic lung injury. BNF also rescued CYP1A2-null mice from oxygen-mediated lung injury. Extent of lung injury positively correlated with levels of hepatic and pulmonary F2 isoprostanes, as determined by GC/MS. In liver and lung, hyperoxia induced CYP1A1 mRNA and protein expression after 2-4 days of exposure, followed by decline at 7 days. BNF-exposed animals showed induction of CYP1A expression at all time points. The protective effects of BNF pretreatment, which was accompanied by induction of CYP1A1/1A2 and decreased levels of F2-isoprostanes lends further credence to the hypothesis that CYP1A1 and 1A2 play beneficial roles in hyperoxic lung injury by CYP1A-mediated detoxification of F2-isoprostanes. These findings have implication for the prevention/treatment of BPD in premature infants. (Supported by K08HL004333, RO1HL070921 and RO1HL087174.)

1775 TRANSPLACENTAL CARCINOGENESIS INDUCED BY MATERNAL EXPOSURE TO DIBENZO[*A, L*]PYRENE: THE ROLE OF CYP 1B1.

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Dibenzo[*a,l*]pyrene (DBP) is one of the most potent of the class of environmental carcinogens known as polycyclic aromatic hydrocarbons (PAHs). In previous studies, administration of DBP to pregnant mice as a single oral dose of 15 mg/Kg on day 17 of gestation caused high mortality in the offspring from an aggressive T-cell lymphoblastic lymphoma. In addition, all mice that survived to 10 months of age exhibited lung tumors with high multiplicity. Expressed Cytochrome P450 (CYP) 1b1 in mice and 1B1 in humans exhibits high activity toward metabolic activation of DBP. In the present study, we bred female and male mice heterozygous for the targeted disruption of the *cyp1b1* gene and examined the effect of gene dosage on DBP transplacental carcinogenesis. Dams were treated with 1 or 15 mg/Kg of DBP or 50 mg/Kg of benzo[a]pyrene (BP). None of the *cyp1b1*-null offspring developed lymphoma. However, the wild-type and het siblings born to mothers given the high dose of DBP exhibited significant mortalities between 10-30 weeks of age. At 10 months of age, mice from all groups exhibited lung adenomas or carcinomas (9.5, 40.3, 25.6 and 100% for control, BP, 1 mg/Kg and 15 mg/Kg DBP, respectively). As expected, the *cyp1b1* status of offspring did not alter BP-dependent carcinogenesis. Conversely, at the low dose of DBP, *cyp1b1* status altered the incidence of lung tumors (19.0, 27.8 and 28.6 for nulls, hets and wild-type, respectively). However, at the high dose, tumor multiplicities in offspring that were *cyp1b1* wild-type (9.3) and heterozygous (9.5) were nearly twice that of the null offspring (5.0). These data confirm that *cyp1b1* bioactivation of DBP occurs in fetal target tissues, primarily the thymus with lung as a secondary target.
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1776 THE CHEMICAL SECURITY ANALYSIS CENTER: ASSESSING THE HAZARD OF CHEMICAL TERRORISM.

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The Department of Homeland Security has been tasked with developing and implementing a capability for providing chemical threat awareness and assessment as applied to issues related to homeland security. The Chemical Security Analysis Center (CSAC) was established to meet these needs and to serve as the central repository of chemical threat characterization data, including data related to toxic industrial chemicals, chemical warfare agents, pesticides, and pharmaceuticals. The CSAC focuses on three aspects: 1) development of knowledge management capabilities related to chemical threats; 2) science based reach back capability; and 3) science and technology based assessments of specific hazards and threats. Using risk based techniques, the CSAC develops toxicologically informed assessments of the hazards posed by chemical threat agents. To support this, available toxicological information is being evaluated to derive new human toxicity estimates for some high risk compounds for which appropriate toxicity estimates do not exist. To guide medical mitigation decisions, the CSAC conducts comprehensive population threat assessments to determine the expected public health impacts of a chemical attack. Plausible, high-consequence scenarios are modeled to predict population exposure and toxic effects for a given chemical, target, dissemination method, route of exposure, and amount of chemical released. Physical properties, mechanism of action, toxicity, and environmental persistence of the chemical are taken into account along with the likelihood of encountering the threat. Classes of chemicals that have been assessed are low volatility nerve agents, pulmonary agents, vesicants, and blood/metabolic agents. The CSAC is also assessing potential disruption due to release of toxic chemicals following attacks on chemical facilities. This involves a complete assessment of chemical dispersion models and a rational way forward on the development of inherently safe technologies that may reduce reliance on toxic chemicals within the industry.

1777 A REGIONAL ASSESSMENT OF PEDIATRIC TOXICANT EXPOSURES OF CURRENT CONCERN.

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The Pediatric Environmental Health Specialty Unit (PEHSU) is a U.S. CDC (ATSDR)/EPA-funded service that provides free telephone consultation on child health toxicant exposure concerns from health care professionals, public health agencies, and the public. Federal region-specific PEHSUs form a network of academic-based U.S. centers. The Region 10 PEHSU serves the states of Alaska, Washington, Oregon, and Idaho and is comprised of University of Washington faculty with training in toxicology, pediatrics, industrial hygiene, neurobiology and public health. In 2006 an Access database was established to track the activity of the NW PEHSU. Quarterly output from this database provides a convenient way to assess the public's perception of regional toxic exposures of concern for children. Between April 2006 and June 2007 the NW PEHSU received 174 initial contacts, with quarterly call volume increasing by 60% over this timeframe. Most inquiries were made by health professionals (61%) and parents (26%). A total of 34 primary substances of pediatric toxicity concern were reported. The majority of these toxicants fall into four categories: metals (32%), pesticides (17%), organic solvents (17%), and indoor air pollutants (17%). Within these categories, lead (16%), benzene (12%) and fungus/mold (16%) predominate as single agents of concern for pediatric toxicity. Childhood exposure events reported to PEHSU were more likely to occur in the home (77%) or school (15%) rather than stem from the parent's workplace (5%) or a public area (1%). Calls to NW PEHSU are increasingly concerned with exposure to environmental agents during pregnancy. PEHSU services would benefit from: (1) more published epidemiology and toxicology research focused on environmental exposures during pregnancy; (2) efforts to improve PEHSU representation beyond Washington State; and (3) the ability to provide in-person consults, rather than phone counsel, for complex cases involving exposure monitoring and health consultation data.

1778 FUNGAL SPORES – A NEW CRITERIA DOCUMENT FROM THE NORDIC EXPERT GROUP.

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The Nordic Expert Group produces scientific bases for setting occupational exposure limits in the Nordic countries. The documents may be downloaded from www.nordicexpertgroup.org. This paper summarises the conclusions in the document on fungal spores (Eduard 2007).
Fungal spores are ubiquitous in the environment. Exposure levels in workplaces where mouldy materials are handled are much higher than in common indoor and outdoor environments. Febrile attacks of organic dust toxic syndrome (ODTS) and hypersensitivity pneumonitis may occur after episodes with excessive exposure. In common indoor environments irritation symptoms have been ascribed to fungi at much lower exposure levels, especially in damp buildings.
The specificity of the response to fungi in hypersensitivity pneumonitis and asthma patients has been clearly demonstrated by bronchial provocation with the suspected organisms. In vitro and animal studies have further shown differences in the inflammatory potential of fungal species. In animal studies, non-allergic responses dominated after a single spore dose but allergic responses, especially to viable spores, were also observed. Repeated exposures induced both non-allergic and allergic pulmonary inflammation.
The combined evidence from human challenge and epidemiological studies support fairly consistent lowest observed effect levels (LOELs) of approximately 100,000 spores/m3 for diverse fungal species in non-sensitised populations. The only well designed study on populations exposed to common indoor air indicates a no observed effect level (NOEL) of about 700 spores/m3. A few human challenge studies with Trichoderma harzianum, Penicillum and Alternaria alternata indicate NOELs of 1,000 – 10,000 spores/m3.

Identification of the fungal biota is required in order to document the occurrence of more toxic mycotoxin-containing and pathogenic species before measurements are evaluated.

Reference:
Eduard W. The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals. 139. Fungal spores. Arbete och Hälsa 21 (2006) 1-145.

1779 APPLICATION OF THE THRESHOLD OF TOXICOLOGICAL CONCERN APPROACH TO INHALED MYCOTOXINS.

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The Threshold of Toxicologic Concern (TTC) identifies daily intake levels that are predicted to cause no adverse health effects in humans over a 70-year lifetime for of a range of chemicals. TTC levels are based on known structure-activity relationships and incorporate wide margins of safety; they do not require chemical-specific toxicity data. The lowest TTC is 0.15 μg/day for a class that includes "aflatoxin-like" compounds. Dietary intake of total aflatoxin by Americans would be higher than this TTC—approximately 0.73 μg/day based on food consumption data—if all corn and nut products consumed contained aflatoxin at the 20 ppb FDA action limit (15 ppb for peanuts). Drew and Frangos applied appropriate adjustments to the TTC concept to develop Concentrations of No Toxicologic Concern (CoNTC) to identify the concentration of airborne substances that would pose no adverse health effect to humans exposed continuously over a 70-year lifetime. The point of departure for CoNTC development was the TTC for potentially genotoxic or carcinogenic substances (1.5 μg/day). Adjustment of this TTC with factors for inhalation (and allowing for other exposure sources) produced 30 ng/m^3 as the generic lifetime average CoNTC. We used the CoNTC to evaluate the hazard of airborne mycotoxins. For several mycotoxins, we calculated the smallest number of spores per cubic meter of air required to achieve the CoNTC given known concentrations of mycotoxin in spores reported in peer-reviewed scientific literature. We found that the lowest airborne spore concentration that could produce a mycotoxin concentration equivalent to the CoNTC is 2.5E6 spores/m^3 for fumitremorgen, 3.0E5 spores/m^3 for satratoxin H, 2.0E7 spores/m^3 for trichoverrols A/B, and 6.3E4 spores/m^3 for aflatoxin B1. This analysis confirms that a wide margin of safety remains for individuals exposed to 6.3E4 spores/m^3 (if aflatoxin is present) or 3.0E5 spores/m^3 (if non-aflatoxin mycotoxins are present).

1780 GENOTOXICITY OF PACLITAXEL – RISK ASSESSMENT FROM A MEDICAL DEVICE PERSPECTIVE.

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For the last few decades, Paclitaxel has been used successfully in the treatment of different types of cancers. More recently this drug is now being used in medical devices. Paclitaxel, coated on cardiovascular stents, has been shown to be effective in treating cardiovascular lesions by prevention of arterial restenosis in coronary arteries. However there is scarcity of published literature on its potential genotoxic effects at the low doses used in such medical device applications.

In the present study, Paclitaxel was tested using both in vitro and in vivo test systems under GLP conditions. In the Ames assay, Paclitaxel dissolved in DMSO was tested from 0.001 to 5.0 mg/plate with and without S9 metabolic activation. The results showed no significant increase of revertants in any of the treated plates compared to the controls. Similar negative results were observed in the in vitro chromosomal aberration assay in CHO cells and human peripheral blood lymphocytes at doses ranging from 0.0001 to 250 μg/ml. For in vivo studies, male CD-1 mice were exposed to different doses of Paclitaxel in corn oil by intraperitoneal injection. Bone marrow was harvested at 24 and 48 hours and screened for presence of micronuclei in polychromatic erythrocytes. The results showed that Paclitaxel is not genotoxic at any of the doses ranging from 0.02 to 40 mg/kg. Statistically significant increases in micronuclei frequencies were seen in mice treated only with the highest dose of 80 mg/kg.

The maximum amount of Paclitaxel loaded onto a cardiovascular Stent is 282 μg. If all of the Paclitaxel were to be released at once, the maximum dose to which a patient is exposed is about 4 μg/kg bw. Based on the present mouse study, NOEL is 40 mg/kg which provides a safety factor of 10,000. In summary, Paclitaxel has been shown to be non-genotoxic at concentrations 10,000 times greater than the total drug content of a Stent.

1781 RISK ASSESSMENT OF A SILVER-BASED DEODORANT INGREDIENT - A SIMPLE APPROACH REPLACING ANIMAL TESTING.

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TINOSAN® SDC is an aqueous ionic silver complex intended for use as an antimicrobial active ingredient in deodorant cosmetic products. For application in spray or roll-on formulations, an adequate human health safety assessment dictates assessing hazard from inhalation and topical leave-on routes of exposure. Absence of inhalation LD50, a key toxicity endpoint and the high relevance of the product's efficacy in deodorants versus the intention to minimize additional animal tests led us to adapt the simple alternative approach we will present in this paper.

Published hazard endpoints for silver and silver-based products were taken as suitable surrogates for test results for Ag+ present in TINOSAN® SDC. Estimates for dermal and inhalation exposures were based on representative parameters taken from recognized sources. To evaluate the range of potential risk we calculated and considered both a worst-case exposure scenario and a reasonable but conservative most likely exposure scenario.

Published data indicated silver in the lungs is readily cleared to systemic circulation and does not result in specific toxic effects. Argyria, the most notable effect of chronic exposure to silver in humans is considered the critical endpoint both by inhalation and by skin contact. Safety evaluation was based on the Reference dose for silver of 0.005 mg/kg/d established by the US Environmental Protection Agency to prevent argyria. The risk calculation derived from this benchmark and our exposure estimates gave Margin of Safety values in the range of 125 to 2778. Thus indicating TINOSAN® SDC can be recommended and supported as acceptable for use in the production and marketing of personal care deodorant products. We report this approach as an example of what could be termed an "Intelligent Alternative Risk Assessment" because it replaced new animal testing with critically evaluated published toxicity data and reasonable case exposure estimations to support new applications of a product in a shorter timeframe and with reduced costs.

1782 ASSESSING NASAL TISSUE DOSIMETRY OF NAPHTHALENE WITH A HYBRID CFD-PBPK MODEL.

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Naphthalene is an important intermediate in chemical synthesis of phthalic anhydrides, carbamate insecticides, resins and tanning agents; it is a component of petroleum fuels and is used as a moth repellent. Chronic inhalation of naphthalene leads to nasal inflammation, hyperplasia of the respiratory epithelium and metaplasia of the olfactory epithelium in rats at concentrations >10 ppm, the lowest concentration tested. Increased incidences of adenoma in respiratory epithelium (male rat) and neuroblastoma in the olfactory epithelium (female rat) were also noted. Differences between the rodent and human nose in anatomy and biochemistry are important considerations in conducting interspecies extrapolations for a naphthalene risk assessment. As a first step, a physiologically-based pharmacokinetic model was constructed to assess the localized uptake and metabolism of naphthalene in the nasal respiratory and olfactory epithelium. Metabolic constants were calibrated to upper respiratory tract extraction data for naphthalene. Vmax for nasal oxidation was estimated to be 39.5 nmol/min/mL with a Km of 3.0 μM. Steady state naphthalene concentrations were predicted to be 266, 796, 2403 μM in the rat olfactory epithelium and 286, 850, 2557 μM in the rat ventral respiratory epithelium at the cytotoxic (3.4 ppm), and the cytotoxic/tumorigenic exposure concentrations of 10 and 30 ppm, respectively. At 3 ppm and 150 ml/min, the percent extraction for naphthalene in the upper respiratory tract is ≈ 40%. In the absence of naphthalene metabolism, the model predicts that the percent extraction in the URT is reduced to ≈ 23 %. In contrast, the difference in extraction with and without naphthalene metabolism is predicted to be less than 2% at 30 ppm. This model will be extended to the human to provide the basis for rodent to human extrapolation to assess risk from naphthalene exposure.

1783 CHRONIC NONCANCER TOXICITY REFERENCE VALUES FOR USE IN ETHYLBENZENE RISK ASSESSMENTS.

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Existing noncancer reference concentration (RfC) and reference dose (RfD) values for ethylbenzene (EB, CAS RN 100-41-4) from U.S. EPA's Integrated Risk Information System (IRIS) were derived in 1991 and 1988, respectively, and are both based on relatively dated studies in which U.S. EPA had placed low confidence. These studies have been superceded by more recent developmental toxicity and oral subchronic studies. In addition, the overall database for testing of EB, in which U.S. EPA also placed low confidence, is now considerably more extensive, with the additional conduct of a multigeneration reproduction study, chronic toxicity studies, neurotoxicity studies, and ototoxicity studies, as well as pharmacokinetic studies and development of physiologically based pharmacokinetic (PBPK) models. Potential toxicity reference values were based upon liver effects observed in chronic inhalation and subchronic oral studies, and upon ototoxicity observed in a

subchronic inhalation study. Benchmark dose (BMD) values for the toxicity reference values are based on dose-response analyses of PBPK-derived internal doses and appropriate uncertainty factors of 30 to 300 for ototoxicity, 30 for chronic liver effects and 300 for subchronic liver effects. A range of UFs for ototoxicity were considered due to differences in interpretation of the biological significance of specific endpoints (i.e., measured effects on hearing or morphological changes in the cochlea). Toxicity reference values were determined using the human PBPK models to perform interspecies and dose-route extrapolations. The additional information and techniques currently available for RfC and RfD determination allowed the development of new proposed toxicity reference values with greater confidence than could be associated with the existing IRIS values. Based on these analyses, a noncancer RfD of 0.5 mg/kg/day and a noncancer RfC of between 0.3 and 0.8 ppm are recommended. Confidence in these reference values is considered medium-to-high.

1784 MODE OF ACTION (MOA) EVALUATIONS AND DERIVATION OF A CANCER REFERENCE VALUE FOR ETHYLBENZENE.

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Ethylbenzene (EB) is carcinogenic in animals following lifetime exposures to high vapor concentrations. Tissue sites with increased tumor incidence following exposure to EB include kidney (rat), testes (rat), lung (mouse), and liver (mouse). Data on cancer MOA for each tissue site was examined using the modified Hill criteria for causation. A direct mutagenic MOA for EB is not supported by available data for any of the observed tumor types. Instead, EB appears to exert its carcinogenic effects via nongenotoxic MOAs. Based on these MOAs, three of the rodent tumor types were considered not relevant to human health due to qualitative species differences. Exacerbation by EB of chronic progressive nephropathy, a pathway that is considered to have no relevance for extrapolation to humans, is postulated as the MOA underlying the development of rat renal cancer. Rat Leydig cell tumors were not considered relevant to humans based on several qualitative criteria. Liver tumors in mice likely involve a phenobarbital-like induction, which is not considered to be a relevant MOA for liver tumors in humans. The lung tumors produced by EB in male mice, however, are assumed to be relevant to human health. Increases in regenerative cell proliferation, due to the formation of reactive metabolites (catechols, quinones), are postulated to play a key role in the mouse lung tumors. A PBPK model was used in the dose-response assessment to account for some of the important quantitative differences between mice and humans with respect to the formation of reactive metabolites in lung tissue. The multistage model was used to estimate a point of departure (POD), as well as upper and lower confidence limits. Based upon a nongenotoxic MOA for mouse lung tumors, the dose-response relationship below the point of departure was assumed to be nonlinear. A cancer reference value of 0.80 ppm (LCL=0.48; UCL=1.1 ppm) was derived based upon an uncertainty factor of 300 applied to the POD for mouse lung tumors. Medium-to-high confidence is placed in this reference value.

1785 A WEIGHT OF EVIDENCE METHODOLOGICAL APPROACH FOR DERIVING EVIDENCED-BASED RFCS FOR GLUTARALDEHYDE: A CASE STUDY.

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There are a number of examples where the USEPA has derived inhalation reference concentrations (RfCs) for specific chemicals that are several orders of magnitude lower than widely accepted occupational exposure limits (OELs). Glutaraldehyde is a case in point where OELs globally are at or near the TLV-C of 50 ppb but USEPA proposed occupational and residential inhalation RfCs ranged from 5-320 ppt. The disparity can be attributed in part to the lack of an integrated assessment of all relevant animal and human data (i.e. a weight of evidence (WOE) methodology). We propose the use of a WOE approach for noncancer risk estimates to validate standard default assumptions used in the calculation of RfCs as a method to improve certainty in the risk estimates. A WOE assessment was conducted for glutaraldehyde which integrated: 1) the known species differences in upper respiratory tract (URT) physiology, 2) benchmark dose modeling (BMD) to refine the point of departure (POD) for URT irritation rather than the NOAEL/LOAEL approach, 3) incorporation of relevant animal/human studies and 4) data from more than 30 years of human use experience to derive an evidenced-based inhalation RfC. BMD modeling estimated the POD (BMDL) for URT irritation at 30 ppb instead of 62 ppb as determined by the lowest NOAEL. Conversion to a HEC predicted humans to be more sensitive than rodents; however, the WOE indicated that humans are likely less sensitive. Thus, conversion to a HEC will clearly overestimate the risk to humans by a significant margin. The WOE further supported an interspecies UF of 1x based on evidence that the rat is more than or equally as susceptible to glutaraldehyde as are humans. An additional intraspecies UF was not warranted since human data in a sensitive subpopulation were available for comparison with the animal data. The WOE assessment indicates that an RfC of 30 ppb more appropriately estimates a safe exposure for humans, including sensitive subpopulations, from glutaraldehyde-induced URT irritation.

1786 DETERMINATION OF A DICHLORVOS-SPECIFIC ACUTE INTERSPECIES UNCERTAINTY FACTOR.

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Uncertainty factors are used in non-cancer risk assessments to account for potential inter- and intra-species sensitivity differences in response to chemical exposures, with a default 10-fold value for each. In the case of dichlorvos (DDVP), there is a large body of relevant human data which can be used to address these extrapolation issues. Previous work comparing data of all durations of exposure collected in animals and humans of all ages, determined that 1 is an appropriate interspecies uncertainty factor for DDVP. The present analysis was undertaken in response to questions concerning occupational exposures and risk. It focuses specifically on acute exposure studies in adults (all species) and includes studies that were not available for and/or utilized in the previous interspecies assessment. Published literature and unpublished data generated in support of product registrations were reviewed, appropriate acute exposure studies with useful dose-response information were identified, and selected data were analyzed to assess DDVP-specific acute interspecies sensitivity differences. The critical effect-response indicator was red blood cell (RBC) cholinesterase inhibition. Levels of RBC cholinesterase inhibition were plotted versus the dose and duration of exposure producing that effect. The data demonstrate that DDVP doses producing specific levels of inhibition in humans following acute exposures are virtually identical to the doses eliciting the same inhibition levels in animals exposed acutely. There is thus no apparent difference in the extent of RBC cholinesterase inhibition following acute DDVP exposure that can be attributed to species sensitivity differences. The data indicate that the acute interspecies uncertainty factor should be 1 when RBC cholinesterase is the endpoint of concern.

1787 RECENT STUDIES OF THE HEALTH EFFECTS OF MANGANESE AND THE IMPLICATIONS FOR THE REFERENCE CONCENTRATION (RFC).

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In 1993, US EPA calculated a Reference Concentration (RfC) of 0.05 $\mu g/m^3$ for manganese based on observations of subclinical neurological effects in workers exposed for an average of 5.3 years. The geometric mean exposure, 150 $\mu g/m^3$ respirable manganese, was considered the LOAEL, and uncertainty factors (UFs) were applied to account for sensitive populations, database limitations, a LOAEL, subchronic exposure, and potential differences in the toxicity of different forms of manganese. Since that time, several epidemiology studies examining exposure to manganese dust and neurological effects, which are considered the most sensitive effects, have been published that may bear on the RfC. For this analysis, we identified nine occupational studies with chronic exposure durations (*i.e.*, greater than 7 years, based on 10% of a 70-year lifetime exposure) that examined subclinical neurobehavioral effects – predominantly effects on motor function (typically visual reaction time, hand-eye coordination, and hand steadiness). We assigned NOAELs and LOAELs as the mean of the exposure group when available (otherwise we used the median or geometric mean). Using this criterion, LOAELs ranged from 100 to 62,500 $\mu g/m^3$ and NOAELs were all approximately 60 $\mu g/m^3$. Overall, these studies provide sufficient evidence to support the use of a NOAEL and a chronic study, eliminating the need for LOAEL and subchronic UFs. In addition, we analyzed recent animal toxicological studies involving developmental exposures. These studies provide evidence to suggest that developmental effects from inhalation of manganese are not more sensitive endpoints than neurological effects, and that a UF for a lack of developmental studies is unnecessary. We conclude that using data from recent epidemiology and toxicology studies leads to an RfC that is at least 10-fold less stringent than the present value.

1788 HUMAN SAFETY RISK ASSESSMENT OF A NOVEL POLYMERIC MATERIAL.

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A new high molecular weight polymer has been developed for use in an innovative product with significant dermatologic exposure. In order to support the use of this novel material, a human safety risk assessment was conducted using an exposure-based risk assessment approach. All constituent monomer impurities were also evaluated for their potential to cause systemic and local health effects. The high molecular weight of the polymer (>1 million) precludes any potential for systemic

bioavailability following dermal exposure. Therefore the risk assessment focused on residual low molecular weight monomer impurities extractable from the polymer. A highly conservative assumption of potential monomer exposure was based on amounts extractable by organic extraction (185 +/- 55 ppm). No monomers were detected in saline extracts (detection limit 4 ppm). Using published NOELs for the monomers, margins of safety were calculated; all were in the range of 2 to 300. Human Repeat Insult Patch and 21-day Cumulative Irritation tests with the neat material and the finished product confirmed there was no evidence of irritation or allergy associated with this material. Given the absence of systemic toxicity from the polymer, the margins of safety against highly conservative and unrealistic potential monomer exposures, and the absence of allergy and irritation effects, there are sufficient data to support the safe use of this new polymer.

1789 APPLICATION OF THE 10% MINIMUM ELICITATION THRESHOLD TO QUANTITATIVE ASSESSMENT OF DERMAL SENSITIZATION RISK TO HEXAVALENT CHROMIUM USING THE REPEAT OPEN APPLICATION TEST IN HUMANS.

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Hexavalent chromium (CrVI) is a chemical component of various wood preservative formulations. The Agency examined exposures and risks from the proposed residential use of wood treated with chromium containing wood preservatives, including children in contact with playground equipment. Particular concern was raised with respect to the dermal sensitization potential of CrVI from repeated dermal contact. In 2004 the Agency sought expert advice from the FIFRA Science Advisory Panel (SAP) and the Agency's Science Policy Council for development of a scientifically sound approach to quantitative assessment of dermal sensitization risk from exposure to CrVI. From these consultations, a level of concern (sensitization Reference Dose, or S-RfD) of 0.009 µg Cr(VI)/cm2 was developed from the study of Nethercott et al. (1994) using the 10% Minimum Elicitation Threshold (MET) value of 0.089 µg Cr(VI)/cm2 in that study and an uncertainty factor of 10x for interspecies extrapolation. As part of the SAP report in 2004, the Panel suggested that a Repeat Open Application Test (ROAT) study could be conducted to better represent real-life exposures to treated wood containing Cr(VI) for refinement of this risk assessment. Such a study using both Cr(VI) and a wood treatment solution containing both copper and Cr(VI) was submitted to the Agency and reviewed in 2006. Based on Agency review of the ROAT study as well as the result of peer review by the Agency's Human Studies Review Board (HSRB), a value of 0.092 µg Cr(VI)/cm2, representing a 10% MET, was selected for the dermal sensitization level of concern of Cr(VI) used as a wood preservative. The 10x uncertainty factor was removed on the basis that the Agency obtained surface residue data for Cr(VI) and the ROAT data provided a response level using a non-occluded testing technique. Disclaimer: This abstract does not represent official EPA policy.

1790 RISK ASSESSMENT OF ALLERGIC CONTACT DERMATITIS DUE TO DERMAL EXPOSURES TO HEXAVALENT CHROMIUM.

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We conducted a recent study of allergic contact dermatitis (ACD) from repeated open application tests to two forms of hexavalent chromium [Cr(VI)]: potassium dichromate (PD) and acid copper chromate (ACC). Differences in the solutions were pH, chromate form, and presence of copper and potassium; but concentrations of Cr(VI) were the same for each. Challenges were conducted on individuals who are allergic to Cr(VI), and the responses were elicitation of ACD and mild transient irritation. The conditions of the challenges were designed to represent possible occupational and environmental exposures to Cr(VI) on the surface of freshly treated wood or Cr(VI)-contaminated soil, while posing minimum risk to study participants. The results are of use for dermal risk assessment of Cr(VI). The 10% minimum elicitation threshold ($MET_{10\%}$)—dose level at which 10% of a sensitized population exhibits an allergic response—was calculated using the Benchmark Dose Software. To ensure that the $MET_{10\%}$ values are protective of ACD, responses that were considered to be irritant by the study dermatologist but might have been allergic, considering the dose-response relationships and whether irritancy persisted with continued dosing, were included in the $MET_{10\%}$. The resulting $MET_{10\%}$ values for the study group were 86 ng/cm^2 for PD and 180 ng/cm^2 for ACC. Because the $MET_{10\%}$ of the study population may be different from that of the general population of individuals who are allergic to Cr(VI), weighted $MET_{10\%}$ values were calculated using the relative proportions of patch-test response grades of the study population and general clinical population of North America. The weighted $MET_{10\%}$ values were 270 ng/cm^2 for PD and 196 ng/cm^2 for ACC and can be used as skin reference doses (S-RfDs) for Cr(VI) in treated wood, on surfaces, or in environmental media such as soil. The concentration of Cr(VI) in soil protective of ACD using the S-RfD for PD is 1,400 mg/kg and that on ACC-treated wood is 200 ng/cm^2.

1791 RISK ASSESSMENT FOR ARSENIC ON INDOOR SURFACES.

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Standard approaches for assessing human health risks for chemicals in soil, air, groundwater, and surface water were established more than 20 years ago with the advent of EPA's Risk Assessment Guidance for Superfund and predecessor guidance. Approaches for assessing the risks associated with exposure to chemicals on indoor building surfaces have not been formalized, and only a few example models exist arising from cleanup at the World Trade Center following 9-11, in response to Homeland Security Presidential Directives, and from efforts by California's Department of Toxic Substances Control and branches of the U.S. military. The presence of trace amounts of arsenic on indoor building surfaces of a manufacturing facility was the impetus for developing a site-specific yet generally applicable scheme for determining health risk-based surface cleanup concentrations that would be protective for building occupants. In keeping with EPA guidance, a surface cleanup concentration range for arsenic was developed using both central tendency and reasonable maximum exposure estimates of surface contact frequency, fraction of dust transferred from surface to skin, dermal surface area available for absorption, fraction of available dermal area that contacts the surface, dermal absorption efficiency, dermal surface area available for ingestion, fraction of available dermal area that contacts the mouth, fraction of dust transferred from skin to mouth, the number of hand-to-mouth events, exposure frequency, and exposure duration. A surface cleanup concentration range of 60 µg/100 cm2 to 970 µg/100 cm2 arsenic was developed using this approach. When compared to area-specific building surface concentrations for arsenic, this range assisted in identifying areas within the building that required cleanup. This approach may be an important step in the process of formalizing risk assessment guidance for indoor building surfaces.

1792 ASSESSMENT OF THE CARCINOGENIC RISK ASSOCIATED WITH THE PRESENCE OF NITRATES AND NITRITES IN DRINKING WATER.

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The abuse of nitrogen-based fertilizers and manures in intensive agricultural regions is among the causes of high occurrence of nitrites (NO2-) and nitrates (NO3-) in drinking water. The presence of NO3- and NO2- in groundwater following those pressures represents a potential risk of cancer for exposed populations. Moreover, these natural occurring substances are widely found in food. Although NO3- is the most stable form of these substances, their indirect toxicity is due to their transformation into NO2- in the digestive tract. In presence of N-nitroso precursors (NP) from food, NO2- lead in the acidic stomach to the harmful N-nitroso compounds (NOCs). For instance the NOC dimethylnitrosamine (DMNA) was classified by IARC as probably carcinogenic for human (2A). NO3-/ NO2- have been also classified as 2A in endogenous nitrosability conditions. The aim of this study is to quantitatively estimate the carcinogenic risk for human associated with levels of NO3-/NO2- occurring in some drinking water systems supplied by groundwater in seven areas of Quebec. Those latter are classified as overwhelmed with manure by their government. To that end, we considered a scenario of complete transformation of NO2- into NOCs. Then the additional risk (AR) of some NOCs was estimated using Monte Carlo simulations and a mathematical model as follow: Risk = [Daily intake of NP] × [gastric conc. of NO2-] 2 × [nitrosability rate constant] × time × q1* (cancer potency factor set by USEPA). For instance, the estimations showed that for DMNA, ARDMNA spanned 2 × 10-9 to 6 × 10-8, which is lower than the acceptable AR set by several agencies. Furthermore this analysis also revealed that the levels of NO2- is far the factor that contributes more to the variation of the AR followed by the daily intake of NP (Sponsor: FQRNT)

1793 HEALTH RISK ASSESSMENT OF BISPHENOL A (BPA) IN DRINKING WATER.

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Bisphenol A is the monomer used in synthesis of polycarbonate plastics and epoxy resins, and it has been implicated as an endocrine disruptor. Multi-generation feeding studies in rats and mice at doses spanning five orders of magnitude failed to

identify signs of developmental toxicity or adverse changes in reproductive tract tissues, although signs of reproductive toxicity may be induced after parenteral injection. Data collected in fetal rat testes and epididymes showed a clear monotonic dose-response rather than a J- or U-shape curve both in the numbers of genes and magnitude of individual gene expression. Controlled ingestion in healthy adult volunteers resulted in complete recovery of the administered dose in urine as the glucuronide at 96 hours, with no parent BPA detected in plasma. The extensive BPA glucuronidation following ingestion is not seen after injection. Only parent BPA binds estrogen receptors. The lack of carcinogenic activity in well-designed and well-conducted studies in rats and mice suggest BPA is not likely to be carcinogenic to humans. The 5 mg/kg-day NOAELs identified for systemic toxicity in rats and mice were lower than oral NOAEL and/or BMDL10 values for reproductive endpoints. Thus, generalized systemic toxicity of ingested BPA represents the appropriate point-of-departure. Using U.S. EPA uncertainty factor guidance, application of 10-fold inter- and intraspecies factors and a 3-fold database uncertainty factor (to account for the lack of rigorous transplacental BPA bioassays and comprehensive neurobehavioral and immunotoxicologic evaluations of BPA by relevant routes and doses) to the 5 mg/kg-day NOAEL results in an oral RfD of 0.016 mg/kg-day. Assuming the 70 kg adult consumes 2 liters of water each day and adopting the default 20% U.S. EPA drinking water relative source contribution yields a 100 µg/L BPA Total Allowable Concentration.

1794 THE POSSIBLE COMMUNITY HEALTH HAZARDS POSED BY EMISSIONS FROM AUTOMOBILE TRAFFIC TUNNELS: A REVIEW OF THE LITERATURE.

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Increased construction of urban traffic tunnels during past several decades has raised new questions about human health effects of traffic emissions. Emissions discharge from portals or exhaust vents has the potential to adversely affect community residents. In addition, tunnel workers are exposed to such atmospheres. We reviewed >300 papers related to tunnels discussing in-tunnel measurements, measured and modeled emissions from portals and ventilation stacks, and subsequent dispersion. Special emphasis was given to carcinogenic chemicals and those associated with cardiovascular and pulmonary health risks. Chemicals were chosen based on available measurements, and those identified in epidemiological and toxicological studies as having potential health effects. Chemicals likely contributing the most health risk are the 17 priority PAHs, carbonyl compounds (i.e. formaldehyde, 1,3-butadiene), carcinogenic or neurotoxic metals (Cr, Ni, Mn, Pb, Mn), CO, NOx, and SO2. Exposure assessment for tunnel emissions is complicated by traffic patterns, vehicle fleet composition, local meteorology and topography. Despite this, most tunnel environments appear to be safe for commuters and tunnel workers for acute exposures to most chemicals. Data on chronic health-effects of workers and nearby residents are extremely limited, but indicate minimal impact by the presence of tunnels, and can even improve local air quality. The minimal available information, particularly for chronic exposures, should be supplemented by new studies. The most important characteristics for such studies are: 1) up-to-date in situ measurements at the tunnel of interest, 2) speciated gas and particle phase measurements, in particular size-fractionated PM, 3) must account for background concentrations.

1795 A PROTOCOL FOR DETERMINING CLEAN-UP STANDARDS AT SITES CONTAMINATED WITH LOW TOXICITY BARITE (BARIUM SULFATE).

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Although different barium compounds are known to exhibit vastly different toxicities, cleanup standards for barium in soil or sediment at environmental corrective action sites are typically based on total barium concentration without regard for the specific barium compounds present. Barite (barium sulfate) is a naturally-occurring mineral form of barium that is widely used in drilling mud for drilling oil or gas wells. As a result, many petroleum exploration and production (E&P) sites and other sites where drilling mud has been handled or processed exhibit elevated concentrations of barium in the soils. Barite is insoluble in water and is poorly absorbed from the intestine, and, as a result, is essentially non-toxic.
Based on a comprehensive literature review of barite toxicity, the Texas Commission on Environmental Quality (TCEQ) has recently agreed that the insoluble barium compound barium sulfate (i.e., barite) is not a compound of concern with regard to human health, thereby eliminating the need for cleanup standards or site remediation for this compound. The TCEQ still requires that their barium cleanup standards be applied to any soluble barium compounds that may be present at a site. To allow the application of barium cleanup standards to soluble barium compounds while excluding barium sulfate, we have developed a protocol to measure the concentration of soluble barium in soil or sediment samples that may contain both barium sulfate and soluble barium compounds. In this paper, we present (1) a review of the toxicity and regulation of soluble barium compounds and insoluble barium sulfate, and (2) a description of our protocol to measure the concentration of soluble barium compounds in soil and sediment samples and the validation of this protocol.

1796 VAPOR INTRUSION TO INDOOR AIR AT A SITE WITH POLYCYCLIC AROMATIC HYDROCARBONS IN SHALLOW SOIL.

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Site A is a coastal site with polycyclic aromatic hydrocarbons (PAHs) in soil due to deposition from historical operations and subsequent use of contaminated soil as fill. Other contaminants such as benzene are also present. USEPA and CalEPA have identified several PAHs as chemicals to consider for the vapor intrusion to indoor air pathway (e.g. naphthalene which CalEPA treats as a carcinogen). This paper presents the approach used to evaluate the indoor air pathway for PAHs at Site A given challenges such as shallow groundwater, contamination in shallow soil, and complexities in soil gas sample collection and analyses for PAHs. Shallow soil gas samples were analyzed for naphthalene by three EPA methods (TO-15, TO-17, and 8260B). Six other PAHs (e.g. chrysene) not commonly analyzed for in soil gas but identified in vapor intrusion guidance were analyzed by TO-13 and TO-17 which use sorbent technology. None of these six PAHs was detected. While naphthalene is present in soil at levels exceeding risk-based concentrations for the soil ingestion and ambient air pathways, and the levels in soil indicated a potential concern for vapor intrusion, it was detected in soil gas at only one location. This location did not correlate to known high detections in other media. Although detection limits were less than the risk-based concentration, only TO-15 and TO-17 detected naphthalene. Naphthalene concentrations measured by TO-15 and TO-17 ranged roughly two-fold indicating either method would have been acceptable at this site. Johnson and Ettinger (J&E) modeling with DTSC default parameters estimated indoor air risk from naphthalene at 4E-6 and 2E-6 for residential and industrial receptors. J&E modeling using site-specific soil data estimated risks approximately one order of magnitude less. Overall, while naphthalene was a risk driver based on maximum projected cumulative cancer risk, soil gas investigation identified only one location where it posed a potential indoor air risk.

1797 CHARACTERIZATION OF CANCER RISK ASSOCIATED WITH MEASURED AIRBORNE BENZENE LEVELS IN THE STATE OF FLORIDA.

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Ambient airborne benzene comes from multiple sources, including industrial, fuel station, and automobile emissions. Chronic occupational exposures to elevated levels of benzene are associated with leukemic cancer risk, particularly acute myeloid leukemia (AML), though epidemiologic evidence for environmental exposures is lacking. This investigation used historic air monitoring data from six counties in the State of Florida to characterize the cancer risk from airborne benzene concentrations utilizing current Federal and State regulatory risk characterization methodologies, and a comparative risk analysis based on occupational epidemiologic evidence. Airborne benzene samples were collected from 24 air toxics monitoring sites in Broward, Duval, Orange, Miami-Dade, Hillsborough, and Pinellas counties. From 2003 to 2006, air samples (n = 3,794) were collected using 8, 12, and 24 hr samples with sub-ambient pressure canister collectors consistent with EPA benzene methodology protocols 101 and 176. The mean benzene concentrations, by site, ranged from 0.18 - 3.58 ppb. Using a risk calculation methodology consistent with the EPA and the Florida Department of Environmental Protection (FLDEP) guidelines, the resulting cancer risk estimates ranged from 4.37 x 10-6 to 8.56 x 10-5, which exceed the FLDEP's acceptable cancer risk level of 1 x 10-6 for all monitoring sites. The cumulative lifetime exposures were calculated in ppm-years, by site, and ranged from 0.036 - 0.702 ppm-years. A comparative analysis with available epidemiological literature revealed that the association between benzene exposure and cancer risk is related to cumulative exposure in excess of 1 ppm-years. The results of this investigation indicate that it is unreasonable to expect additional cancer cases in Florida residents due to measured ambient airborne benzene levels, despite the fact that all regulatory risk calculations exceed acceptable cancer risk levels in the State of Florida.

1798 COMPARISON OF CONGENER-SPECIFIC AND AROCLOR QUANTITATION METHODS FOR ASSURING REMEDIATION OF POLYCHLORINATED BIPHENYLS AND ASSOCIATED DIBENZOFURANS AT A FORMER WASTEWATER TREATMENT PLANT.

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Elevated polychlorinated dibenzofurans (PCDFs) and associated dioxin toxic equivalents (TEQ) in soils were uniformly co-located with polychlorinated biphenyls (PCBs, Aroclor 1248 pattern) at a residential redevelopment site in California that decades prior housed a publicly operated wastewater treatment plant. Since PCB mixtures characteristically contain PCDFs, we proposed that setting target cleanup levels based on PCB congeners correlated with PCDF-TEQ in soil may avert substantial additional analytical costs for site-wide characterization of dioxins (at 4- to 6-times the cost of the PCB congener assay). Twenty soil samples were taken from areas with high (> 10,000 ppb), moderate (500 - 9,999 ppb), and low levels (< 499 ppb) of total PCBs. Different laboratories analyzed split samples for 1) PCBs as Aroclors by EPA 8082, 2) congener-specific PCBs using the moderate resolution method of DeCaprio et al. (2000), and 3) congener-specific dioxins using EPA Method 1613. EPA 8082 and DeCaprio methods gave consistent total PCB results. Strong and consistent correlations were found between soil PCB congeners or homolog series and similarly chlorinated PCDFs/TEQ. The PCB congener data allowed calculation of a threshold level for PCBs in soil assuring that no PCB-related dioxins in excess of a target TEQ level would be present in site soils. The congener-specific PCB data also revealed strong and consistent correlations between co-located PCDFs/TEQ and content of associated PCB precursors. We conclude that the DeCaprio method is far more cost-effective than applying EPA 1613 at sites with relatively uniform soil PCB contamination and provides more specific and useful information for evaluating the probable source(s) and toxicity of PCBs and dioxins in soils compared to the EPA 8082 Aroclor method.

1799 MICROBIAL RISK ASSESSMENT FOR RECREATIONAL USE OF CHICAGO AREA WATERWAYS.

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Exposure to microbiologically contaminated recreational water has been linked to multiple adverse health outcomes including infections of the eyes, ears, skin, and gastrointestinal system. This analysis was conducted to estimate the risks from recreational use of a waterway receiving treated, but non-disinfected, effluent from a municipal wastewater treatment facility. Waterway samples were collected at locations upstream and downstream of the effluent outfall during dry and wet weather conditions within the recreational season. Samples were submitted for microbiological analysis for organisms that have the potential of producing gastrointestinal illness including: 1) bacteria (E. Coli, enterococci, and Salmonella); 2) protozoa (Giardia and Cryptosporidium); and 3) viruses (total enteric viruses, callicivirus and adenovirus). The geometric mean of the pathogen concentration from either the dry or wet weather sampling events was assumed to represent the expected pathogen levels over the recreational segment of the waterway for that type of weather. A probabilistic analysis was conducted to estimate exposure. Recreational activities considered were canoeing, boating and fishing. Exposure parameters were developed from the primary literature and local use surveys. Since gastrointestinal disease is often spread through secondary contact with infected individuals, the population of interest included the primary receptor in addition to the receptors immediate family. Secondary attack rates were developed for each pathogen from literature sources. Results show the estimated rates of gastrointestinal illness for recreational users is below the EPA recommended level of eight per thousand. A sensitivity analysis suggests that weather type, location along the waterway, receptor activity and ingestion volume all contribute to the probability of developing illness.

1800 HUMAN HEALTH RISKS ASSOCIATED WITH EXPOSURE TO PATHOGENS IN WATERS AND SEDIMENTS OF THE LOWER PASSAIC RIVER.

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Untreated human sewage is often intentionally discharged to surface water during precipitation events via combined sewer overflow (CSO) systems. The purpose of this study was to characterize the microbial contamination and subsequent human health risk associated with exposure to pathogen-contaminated water and sediments near CSOs along the Lower Passaic River. Their potential contribution to the overall pathogen load was assessed in two phases. First, surface water from a discharging CSO was sampled immediately following a rainstorm, and second, pathogen contamination of river sediments was evaluated. Concentrations of fecal coliform, total coliform, fecal *Streptococcus*, fecal *Enterococcus*, *Pseudomonas aeriginosa*, *Staphylococcus aureus*, *Giardia* and *Cryptosporidium* were assessed and a quantitative microbial risk assessment was conducted to evaluate the risk of gastrointestinal illness associated with incidental ingestion of both water and sediment from a single exposure event using multiple exposure scenarios. Water samples were found to contain many bacteria species that are associated with human fecal matter at concentrations several orders of magnitude greater than water quality standards. These water samples also contained *Giardia* and several viral pathogens. Results demonstrate that the estimates of risk for gastrointestinal illness associated with the ingestion of pathogens in river water were significantly elevated; probabilities of illness ranged from 4% to nearly 100% for all scenarios evaluated. Risk estimates associated with ingestion of sediments ranged from approximately 4% to 6% for fecal *Streptococcus* and *Enteroccus. Giardia* risks ranged from approximately 0.1% to 0.4% across all sediment locations where detected. Taken together, this risk assessment suggests that pathogen-contaminated water and sediments near areas of CSO discharge in the Lower Passaic River could pose a health risk to individuals coming into contact with these waters and sediments.

1801 IRON DEFICIENCY AS MODE OF ACTION FOR RAT ORAL CAVITY NEOPLASMS IN 2007 NATIONAL TOXICOLOGY PROGRAM DRINKING WATER STUDY OF HEXAVALENT CHROMIUM.

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In 2007, the National Toxicology Program (NTP) completed a 2-year drinking water study of hexavalent chromium [Cr(VI)] in rats and mice. In the study, dose-dependent increases in oral cavity neoplasms were observed in rats but not in mice, and treatment-related anemia was more severe in rats. Non-neoplastic lesions were not observed in the rat oral mucosa, indicating a Mode of Action (MOA) that does not involve chronic tissue irritation and regenerative hyperplasia. Reported clinical measures implicate multiple etiologic components of oral cavity tumors. Current scientific literature suggests iron (Fe) deficiency as a primary risk factor for oral epithelial changes that may be preneoplastic. As Chromium has been identified to interfere with Fe metabolism, there is a reasonable probability that Fe deficiency is a necessary precursor event for rat oral cavity tumor formation. To characterize the relationship between Fe deficiency and oral carcinogenesis, the histopathology and hematology profiles reported in the 1996, 1997, and 2007 NTP studies of Cr(VI) were analyzed. Treatment-related anemia was observed for high dose groups (>2 mg/kg-day), with statistically significant decreases in mean cell volume and mean cell hemoglobin, irrespective of whether administration was via feed or water. Further, data for other chemicals in the NTP database specifically associated with oral cavity tumors were screened to identify associations between hematological changes and anatomic pathological outcomes. Only 21 of over 550 NTP studied chemicals caused oral cavity tumors. A review of these indicates: 1) rats are more susceptible than mice, 2) tumors occur at multiple sites, 3) non-neoplastic lesions are not commonly observed, 4) most chemicals are associated with anemia indicating Fe deficiency as a MOA for rat oral cavity tumors. Research efforts to quantify the dose-response in the low-dose range should focus on metabolic competition between Fe and Cr(VI) and elucidation of its role in the induction of anemia as a precursor event for oral cavity cancers.

1802 CHRONIC PERCHLOROETHYLENE EXPOSURE IN TWO DIFFERENTLY SENSITIVE GROUPS: A META-ANALYSIS.

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Perchloroethylene (PERC) is widely used in dry cleaning, thus exposing workers as well as non-workers. The effects of PERC exposure have been studied and reported in five publications. Because these publications studied different dependent variables, and used different measurement scales, quantitative comparison of the results is difficult. To facilitate comparisons of results, each reported dependent variable was transformed into an "effect magnitude" (EM) scale in which when there was no effect of PERC, EM=0.0 and when PERC produced as large an effect as was possible, then EM=1.0.

When all five studies were compared (after transformation) it became apparent that the results were remarkably heterogeneous. The most sensitive study reported effects at exposure levels that were ca. 500 times lower than the least sensitive. Furthermore, the least affected groups of subjects, generally had the highest exposures. Yet, when there were multiple dependent variables per study, the results were remarkably consistent. When studies were sorted into two groups, subjects exposed

in the work-place and those exposed to PERC in their homes, it was apparent that the home-exposed subjects were the most sensitive. The difference between groups could possibly due to a healthy-worker effect, different incentives to appear impaired, undocumented differences in socioeconomic factors or age, different temporal patterns of exposure, different access to health care, etc. The uncertainties about any conclusion involve (a) very few reported studies (b) relatively few subjects per study (c) common methodological problems with all of the studies and (d) usual incomplete reporting of data and methods. If the magnitude of the group effect is reliable, this would be an important finding for regulatory decisions as well as for information about the mechanism by which PERC effects are produced.(This abstract does not reflect EPA policy)

1803 HEALTH OF CATTLE AND WILDLIFE DOWNWIND FROM GAS FACILITIES: THE WESTERN CANADA STUDY.

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The Western Canada Study of Animal Health Effects Associated with Exposure to Emissions from Oil and Natural Gas Field Facilities was begun in 2000 to answer specific questions related to alleged exposure-related averse outcomes in cattle downwind of gas facilities, mostly related to reproduction, calf production and mortality. The study was designed to answer primary hypotheses with sufficient power to resolve the issues and secondary hypothesis with enough power to provide a reasonably authoritative answer. Ultimately, 33,000 cows (which in turn produced 28,000 calves) were followed through two calving cycles in four western Canadian provinces, each individually tracked with herd-level exposure assessment based on passive dosimetry and, for a subset, supplemental fine particulate monitoring. A nested wildlife study was conducted at the same locations using European starlings as an indicator species. With specific anomalies and exceptions noted in the report, there was no overall pattern to suggest substantial adverse effects on cattle reproduction or wildlife. Air contaminant levels were relatively low compared to urban air pollution. The Study was professionally managed by an independent corporate entity created for the purpose, Western Interprovincial Scientific Studies Association, with oversight by a Scientific Advisory Panel. The principal investigator for the Study was Dr. Cheryl Waldner, veterinary epidemiologist at the University of Saskatchewan. Further information is available at: http://wissa.hillandknowlton.ca.

1804 CORTICOSTERONE PRETREATMENT ATTENUATES NEURODEGENERATION AND BLOOD-BRAIN BARRIER DISRUPTION FOLLOWING EXCITOTOXIC DAMAGE FROM KAINIC ACID.

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Glucocorticoids are believed to increase hippocampal susceptibility to excitotoxic injury; however, the mechanisms by which steroids affect neurodegeneration are not well understood. Here, we utilized histological and biochemical methods to examine the interaction of corticosterone (CORT) and kainic acid (KA) on neurodegeneration, blood-brain barrier (BBB) disruption, and glial activation in the hippocampus of C57BL/6J mice. Male mice were implanted with 10, 35, or 100 mg/21 d release corticosterone pellets. After seven days, mice received an intraperitoneal injection of saline or 25 mg/kg kainic acid, were scored for seizures (Racine scale), and were allowed to recover for: 12 or 24 hours (histology); 0, 1, 3, 6, or 12 h (western blot); or 7 d (ELISA). Tissue was prepared for histological analysis of neurodegeneration using the cupric-silver stain, while microglia and astrocytes were visualized using immunohistochemical analysis of Iba-1 and GFAP, respectively. BBB disruption was quantified by western blotting using an anti-mouse IgG antibody. By 12 h following KA, damaged neurons were observed throughout all parts of the hippocampus, and activated microglia and astrocytes were observed in regions displaying neurodegeneration. A five-fold elevation of IgG in the hippocampus was evident six hours following KA injection. CORT alone did not induce seizures, produced no neuronal or BBB damage, and did not exacerbate the injury produced by KA. Pretreatment with CORT attenuated KA-induced neurodegeneration, glial activation, and BBB breach. Analysis of GFAP by ELISA revealed an elevation in protein levels following KA treatment (524.1 +/- 159.6%). CORT dosage and basal GFAP levels followed an inverse relationship (92.3 +/- 5.4%, 60.8 +/- 6.5%, 47.7 +/- 5.2%, of control, respectively). In CORT pretreated animals, KA-induced elevation of GFAP was attenuated (107.1 +/- 15.7, 94.1 +/- 22.4%, 53.4 +/- 7.2, of control, respectively). Considered together, these data suggest high dosages of corticosterone provide protection against KA-induced excitotoxicity.

1805 ACUTE NEUROTOXICITY STUDY OF 1, 2-DICHLOROETHANE (ETHYLENE DICHLORIDE; EDC) *VIA* INHALATION IN F344 RATS.

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The acute neurotoxic effects of EDC were evaluated in F344 rats exposed for 4 hours (h) to target concentrations of 0, 200, 600, or 2000 ppm via whole body inhalation. A functional observational battery (FOB), including motor activity (MA), hind- and forelimb grip performance, rectal temperature (RT), and foot splay, was conducted prior to exposure, ~1h after exposure (day 1) and on days 8 and 15. Neuropathologic evaluation was conducted following perfusion on day 16. Treatment-related FOB observations on day 1 were: decreased resistance to removal, palpebral closure, lacrimation, decreased extensor response and response to noise and pinch, increased urination, defecation, and slight gait incoordination in 2000 ppm rats. Response to noise was decreased in 600 ppm females and urination was increased in 600 ppm rats on day 1. Exposure to EDC did not affect grip performance or foot splay. RT was decreased on day 1 in rats exposed to 600 or 2000 ppm. There was a decrease in total MA and an altered within-session distribution of MA counts in 2000 ppm females on day 1. There were no treatment-related observations or effects seen in RT or MA on days 8 or 15, nor any treatment-related neuropathologic changes in the central nervous system. Treatment-related lesions in the peripheral nervous system were limited to regeneration of the olfactory mucosa 15 days after exposure at all concentrations. To identify a no-observed-effect level (NOEL) and to expand the dose-response range, rats were exposed to target concentrations of 0, 50, 100, or 150 ppm EDC for 8h and 50 ppm EDC for 4h and euthanized 24h later. Histopathologic examination revealed morphologic changes in the olfactory mucosa at concentrations of 100 or 150 ppm. No effects were observed in rats exposed to 50 ppm for 4 or 8h. The NOEL for behavioral neurotoxicity was 200 ppm EDC for 4h, while the NOEL for neuropathology was 50 ppm EDC for up to 8h in F344 rats.
(Funded by the HAP Task Force, a testing consortium of US EDC producers)

1806 NERAMEXANE LACKS INTERACTION WITH DONEPEZIL AND RIVASTIGMINE AT THE ACETYLCHOLINESTERASE LEVEL IN DISCRETE BRAIN REGIONS.

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Cholinergic deficit and NMDA receptor up-regulation are the two major mechanisms involved in Alzheimer's disease. Currently, no single drug corrects both mechanisms, and therefore a combination therapy is considered essential. The present study was undertaken to determine whether neramexane (NMDA receptor antagonist) influences acetylcholinesterase (AChE) inhibition induced by donepezil, rivastigmine or diisopropylphosphorofluoridate (DFP) in specific brain regions (cortex, hippocampus, striatum, and brainstem) of rats. Donepezil, rivastigmine or DFP were administered at doses of 0.75, 0.35, and 1.5 mg/kg, ip, respectively. At these doses, each compound produced approximately 50% AChE inhibition (needed to elevate acetylcholine concentrations to behaviorally relevant levels), and was well tolerated. Neramexane (NER) at therapeutically relevant or greater doses (3.1, 6.2, or 12.4 mg/kg, ip), produced neither AChE inhibition nor any behavioral toxicity. Rats treated with NER in combination with an AChE inhibitor were sacrificed 60 minutes after DFP, 15 minutes after donepezil and 30 minutes after rivastigmine administration (these times were based on maximal AChE inhibition established from time course studies). NER neither inhibited AChE activity nor influenced AChE inhibition by donepezil or rivastigmine. However, in a dose-dependent manner, NER significantly ($P<0.05$) attenuated AChE inhibition induced by DFP. It can be concluded that co-administration of NER does not alter AChE inhibition induced by donepezil or rivastigmine. Supported by Merz Pharmaceuticals.

1807 PERSISTENT EFFECTS OF REPEATED INHALATION OF TOLUENE: 4 WEEKS *VS.* 13 WEEKS.

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Understanding and predicting the extent of neurotoxic damage from repeated exposure to volatile organic compounds (VOCs) is a problem for many EPA programs. Eighty adult, male Long-Evans rats inhaled toluene (0, 10, 100, or 1000 ppm) 6 hr/day, 5 days/week for 4 weeks in a systematic replication of a previous 13 week exposure using the same conditions. Cohort 1 (N=40) was trained to perform

a visual signal detection task to assess learning and sustained attention. Previously, 13 weeks of exposure delayed the acquisition of a lever-press response (autoshaping) in all toluene-exposed groups. In the current study, rats exposed for only 4 weeks showed no delay in acquiring a lever response task. 4-week exposed rats also did not show impairment in learning a subsequent visual discrimination task, whereas rats that were exposed to 1000 ppm toluene for 13 weeks were slow to learn the visual discrimination task. No further deficits were observed in either the 4-week or 13-week groups of rats in subsequent behavioral training or testing. Retinal function was assessed in Cohort 2 (N=40) approximately 2 weeks after termination of exposure by recording electroretinograms (ERGs) from dark-adapted, anesthetized rats. The amplitude of ERG B-waves was slightly reduced in rats exposed to 1000 ppm toluene, an effect similar in direction but of lesser magnitude than that observed after 13 weeks of exposure. These results suggest that neurotoxic effects of toluene appear and increase as exposure is increased from 4 and 13 weeks. Given the rapid tissue clearance of toluene, these results are consistent with a progressive impairment with ongoing repeated exposures. [This abstract does not necessarily reflect EPA policy.]

1808 EFFECT OF ACRYLONITRILE INTOXICATION ON BRAIN ENERGY METABOLISM.

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Acrylonitrile (AN) is an organic compound produced in large quantities by the chemical industry and is acutely toxic. One mechanism proposed to explain the toxicity of AN is metabolism by P450 into cyanide (CN). Although blood and brain levels of CN in rats following an LD90 dose of AN are consistent with acute toxicity, blocking CN formation with P450 inhibitors does not prevent lethality. Another mechanism implicated in toxicity is covalent binding of AN to cysteine residues in tissue proteins. Previous work in our laboratory has shown that AN can irreversibly inactivate the catalytically active cysteine-149 in glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Inactivation of GAPDH by AN would be expected to impair glycolytic ATP production and, when coupled to the inhibition of mitochondrial ATP synthesis by the AN metabolite CN, would result in metabolic arrest, particularly in brain. In this study we have measured the energy metabolites PhosphoCreatine (PCr), ATP, ADP and AMP by HPLC and compared their levels at the instant of death in brains of rats treated with an LD90 dose of AN vs. controls. Two methods of rapid brain freezing in liquid nitrogen were used: Funnel Freezing (FF) and Head Immersion (HI). AN administration resulted in large decreases in PCr of 74% (FF) and 80% (HI) but relatively minor decreases in ATP of 5% (FF) and 21% (HI) and Energy Charge of 6% (FF) and 10% (HI). These results are not consistent with brain metabolic arrest being the mechanism of acute AN lethality.

1809 SCREENING THE ACTIVATION STATE OF MULTIPLE PHOSPHOPROTEINS REVEALS THE *IN VIVO* IMPACT OF STRESSORS ON BRAIN SIGNALING PATHWAYS CONTROLLING CELLULAR STRUCTURE.

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Stress results in brain plasticity; these changes likely involve transduction of signals in a complex signaling network. We exposed C57Bl6/J male mice to various stressors and examined the changes in the phosphorylation state of multiple proteins to determine how stress affected signaling. Phosphorylation, the dominant mode of post-translation modification involved in cellular signaling, is controlled through the enzymatic action of both kinases and phosphatases. As these enzymes remain active under a variety of post-mortem conditions all analyses were conducted on tissue obtained after microwave euthanasia, a procedure that ensures death within no more than 50 msec. Moreover, because brain temperature of ~ 90oC is achieved all enzymatic activity is destroyed, preserving in vivo phosphorylation state. Mice were exposed to one of the following: (1) a single 2-hr restraint, (2) a single 5-min swim in 4o C water, (3) 3 consecutive 10 min swims at room temper-ature separated by 5-min periods of non-swim, (4) 7 days of continuous exposure to corticosterone through the implant of a 100 mg pellet or (5) 21 consecutive days of stressors 1, 2, or 3. Hippocampus, cortex and striatum were collected following a stress session, at 24 hrs or 1 week later. A large-scale analysis of signaling networks was performed using Kinetworks Phospho Site Screens; this technique involves the use of phosphospecific antibodies to proteins from many of the currently known signaling pathways & allows the simultaneous determination of the phosphorylation status of ~ 80 phosphoproteins. Cell structure alterations characterize brain plasticity; we observed activation changes in multiple phosphoproteins linked to cell structure including the cytoskeletal proteins α- and γ-adducin, GSK3α, & tau. The direction and intensity of the change depended on the stressor and the brain area examined. Our data suggest an examination of the signaling pathways involved in the control of the cytoskeleton may be warranted to increase our understanding of stress-induced plasticity.

1810 EFFECTS OF DIETHYL MALEATE PRECONDITIONING ON THE EXPRESSION OF DNA REPAIR GENES IN MIDBRAIN ELICITED BY OCHRATOXIN-A.

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Ochratoxin-A (OTA) is a mycotoxin and can cause oxidative DNA damage throughout the adult rodent brain. The objective of this study was to elucidate the mechanism of OTA neurotoxicity in the context of oxidative DNA damage and repair. Taking into account that nigro-striatal dopaminergic neurons are especially vulnerable to oxidative stress we focused on substantia nigra (SN) and ventral tegmental area (VTA) of midbrain. Hypothesizing that vulnerability to OTA is associated with deficient DNA repair, we measured acute effects of OTA on the expression of DNA repair genes coding oxyguanosine glycosilase (OGG1) and poly (ADP) ribose polymerase (PARP) and apurinic/apyrimidinic endonuclease (APEX). Mice received a single dose (4 mg/kg, i.p.) of OTA and were sacrificed after 72 hrs. Knowing that diethyl maleate (DEM) at low doses up-regulates OGG1 activity, we explored its potential protective effect. Animals were preconditioned with DEM (1 mmol/kg, i.p.) 6 hrs before OTA administration. In whole midbrain OGG1 gene was down-regulated by OTA, but when DEM was given in the pre-conditioning paradigm, there was marked up-regulation of the gene. Expression of OGG1 gene was markedly down-regulated in both SN and VTA by the pre-conditioning. APEX was up-regulated in VTA following administration of DEM but was down-regulated by DEM pre-conditioning. The biggest contrast between SN and VTA in the profile of DNA repair was in the regulation of PARP. The VTA profile of PARP regulation may provide a clue as to the relative resistance of VTA dopaminergic neurons to oxidative stress. In the case of the SN, pre-conditioning resulted in a down-regulation of PARP gene below control levels. These results provide understanding of the mechanism(s) responsible for the vulnerability of specific populations of dopaminergic neurons that may lead to discovery of new ways to protect against neurotoxicity. Supported by the Department of Defense Grant #DAMD17-03-1-0501 and VA Merit Review Grant to J.S-R.

1811 EFFECTS OF 4-HYDROXY-2-NONENAL ON NEUROTRANSMITTER TRANSPORT AND VESICULAR STORAGE IN RAT STRIATAL SYNAPTOSOMES.

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4-Hydroxy-2-nonenal (HNE) is an α,β-unsaturated carbonyl derivative of the type-2 alkene chemical class and might play a pathogenic role in the synaptotoxicity associated with neurodegenerative conditions such as Alzheimer's disease. In a recent structure-toxicity study (Tox. Sci. 98: 561-570, 2007), we showed that acrylamide (ACR), acrolein and other type-2 alkenes produced in vitro neurotoxicity (synaptosomal dysfunction) mediated by formation of Michael-type adducts with thiolate sulfhydryl groups on presynaptic proteins. In the present research we expanded our structure-toxicity analysis to include HNE. Results show that in vitro exposure of rat striatal synaptosomes to HNE (0.001-10 mM) produced graded, concentration-dependent inhibition of radiolabled dopamine membrane transport (IC50 = 441μM). This effect was accompanied by parallel graded decreases in synaptosomal free sulfhydryl groups (IC50 = 500μM). HNE exposure of synaptic vesicles also resulted in concentration-dependent inhibitions of dopamine uptake and sulfhydryl loss (IC50s = 56μM and 117μM, respectively). Calculations of softness (σ) and electrophilic reactivity (ω) showed that HNE was less electrophilic than NEM and acrolein, but significantly more reactive than ACR. The rank order of these quantum mechanical parameters was closely correlated to the respective second order rate constants (k2) for the reactions of HNE and other conjugated alkenes with cysteine sulfhydryl groups. As further documentation of nucleophile targeting, we determined the ability of N-acetyl-L-lysine (NAL), N-acetyl-L-cysteine (NAC) or alanyl-L-histidine (carnosine, 500 μM each) to prevent in vitro HNE neurotoxicity. NAC prevented HNE-induced synaptosomal toxicity, whereas neither NAL nor carnosine afforded neuroprotection. These findings show that HNE is a moderately soft electrophile that can inhibit nerve terminal processes by forming adducts with nucleophilic cysteine sulfhydryl groups. (Sponsored by NIEHS ES003830-20)

1812 NEUROPROTECTIVE EFFECTS OF MIXED TOCOPHEROLS IN ANIMAL MODELS OF PARKINSON'S DISEASE.

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Parkinson's disease (PD) is a progressive neurodegenerative disorder that affects more than 1.5 million people in the United States alone. A plethora of evidence indicates that oxidative stress leads to the demise of nigral dopaminergic neurons.

Although considerable progress has been made over the past decade in understanding the disease etiology, to date there is no therapeutic strategy for the prevention or treatment of this disease. Vitamin E (α-tocopherol, αT) has been tested both in animal models of PD as well as in human clinical trials, but with equivocal results. Although αT is the biologically most active form of Vitamin E, γ-tocopherol (γT) is the most abundant form found in the U.S. diet. γT is also a stronger antioxidant and anti-inflammatory agent compared to αT. Thus, to determine the effectiveness of γT to protect against dopamine cell death, animals were pre-treated for 2 mons with either a 0.3% mixed tocopherol diet (containing more than 50% gamma-tocopherol) or control diet and subsequently treated with saline, 10mg/kg paraquat (PQ) + 30 mg/kg maneb (MB) twice a week for 6 weeks, or 25 mg/kg MPTP everyday for 5 days. Animals were sacrificed 1 week after the last dose. The mixed tocopherol diet showed a partial protection against decreases in motor activity due to exposure to PQ+MB and MPTP 1 week after the last exposure. Both MPTP and PQ+MB exposure caused a significant decrease in striatal dopamine and metabolite levels in mice on the control diet. However, mice on the mixed tocopherol diet treated with either PQ+MB or MPTP were partially protected against decreases in striatal dopamine and metabolite levels. This data suggests that γT may prove useful in protecting dopamine neurons against oxidative stress generated by exposure to dopaminergic neurotoxicants. Further studies to establish the mechanism of protection of the tocopherols will prove useful in determining the efficacy and target(s) of neuroprotection in PD. Supported by ES005022

1813 PROTEIN MODIFICATION IN RAT STRATIAL SYNAPTOSOMES AS A RESULT OF THE INHIBITION OF DOPAMINE METABOLISM BY PRODUCTS OF OXIDATIVE STRESS.

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Parkinson's disease is a neurodegenerative disorder characterized by cell death of dopaminergic neurons. Current studies have indicated that cell death may originate from increased oxidative stress leading to augmented levels of an endogenous neurotoxin. Within dopaminergic neurons, dopamine (DA) is metabolized to 3,4-dihydroxyphenylacetaldehyde (DOPAL) through oxidative-deamination catalyzed by monoamine oxidase. DOPAL is then primarily oxidized to 3,4-dihydroxyphenylacetic acid (DOPAC) by mitochondrial aldehyde dehydrogenase (ALDH). ALDH can be inhibited by aldehydes derived from oxidative stress, e.g. 4-hydroxynonenal (4HNE), which may impair DA metabolism causing increased concentrations of DOPAL, a potential neurotoxic intermediate. Previous work has established that upon addition of 4HNE to rat striatal synaptosomes a decrease in DOPAC levels and a corresponding increase in DOPAL concentrations are observed. Based on this information, it is hypothesized that DOPAL, generated at aberrant levels due to impairment of DA metabolism, will cause protein modification. This work utilizes an aminophenylboronic acid resin, which has the ability to specifically bind catechol-containing adducts, to isolate DOPAL-modified protein from rat striatal synaptosomes treated with various concentrations of 4HNE. DOPAL-protein adducts were then separated by SDS-PAGE and detected through silver stain. In addition, a novel synthetic procedure for the synthesis of DOPAL has been established which enables a method for obtaining DOPAL without sacrificing animals (i.e. biosynthesis). The data establish the ability of DOPAL to modify proteins thus indicating its potential role in the pathogenesis of neurodegenerative diseases.

1814 HUMAN PROSTAGLANDIN H SYNTHASE (hPHS)-1 AND hPHS-2-DEPENDENT PROTEIN AND DNA OXIDATION CAUSED BY DOPAMINE, ITS PRECURSOR AND METABOLITES.

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hPHS-1 and -2 are implicated in neurodegeneration but the mechanism is unclear. Neurotransmitters such as dopamine (**DA**), its precursor L-dihydroxyphenylalanine (**L-DOPA**) and metabolites dihydroxyphenylacetic acid (**DOPAC**), homovanillic acid (**HVA**) and 3-methoxytyramine (**3-MT**) may serve as substrates in the brain for PHS-catalyzed bioactivation to free radical intermediates, which generate reactive oxygen species (**ROS**), potentially causing oxidative damage to cellular macromolecules (proteins, DNA). Studies using purified PHS or microsomes to compare PHS-1/2 bioactivating potentials are difficult to interpret due to differing isozyme catalytic stability. Alternatively, we have found that CHO-K1 cell lines stably expressing hPHS-1 or hPHS-2 exhibit enhanced cytotoxicity when treated with DA, its precursor or metabolites. Herein, we used these cell lines to investigate hPHS isozyme-dependent protein and DNA oxidation by DA, its precursor and metabolites. Cells were incubated for 6 or 24 hrs with 1000 uM DA, L-DOPA, DOPAC, HVA or 3-MT, with or without exogenous arachidonic acid (**AA**) to modulate hPHS activity. Protein oxidation, quantified by protein carbonyl content, was increased within 24 hr in both hPHS-1 and -2 cells exposed to DA or L-DOPA ($p<0.05$), and further increased by AA addition ($p<0.05$). After a 1-hr incubation, DNA oxidation, quantified by 8-oxo-2'-deoxyguanosine formation, was enhanced in both hPHS-1 and -2 cells by DA, L-DOPA, DOPAC and HVA ($p<0.05$). AA addition caused a further 10-30% increase in DNA oxidation in cells incubated with DA, L-DOPA, DOPAC or HVA. hPHS-2 cells were more susceptible to DNA oxidation than hPHS-1 cells when treated with DA, L-DOPA and HVA ($p<0.05$), despite hPHS-1 cells having about 2-fold higher activity. Thus, hPHS-catalyzed bioactivation of endogenous neurotransmitters, their precursors and metabolites can cause macromolecular oxidative damage that may contribute to neurodegeneration associated with aging. [Support: CIHR, CIHR/Rx&D HRF].

1815 ALTERED GABAERGIC NEUROTRANSMISSION IS ASSOCIATED WITH INCREASED SUSCEPTIBILITY TO NMDA-INDUCED EXCITOTOXICITY IN CYCLOOXYGENASE-2 DEFICIENT MICE.

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While it is known that inhibition of cyclooxygenase (COX)-2, but not COX-1, increases susceptibility to systemically-administered, kainic acid (KA)-induced excitotoxicity, it is unclear if susceptibility to excitotoxins that act through other neurotransmitter receptors is altered by COX-2 inhibition. To further understand the involvement of COX-2 in regulating susceptibility to excitotoxicity, we investigated the effect of COX-2 deletion on N-methyl-D-aspartate (NMDA; a NMDA receptor agonist) and lindane (a GABAA receptor antagonist)-induced excitotoxicity. COX-2-/- mice injected intraperitoneally with NMDA (25-100 mg/kg) exhibited significantly increased median seizure intensity when compared to COX-2+/+ mice. Further, neuronal damage was observed by Fluoro Jade B (FJB) staining in the CA3 region of COX-2-/- mice exposed to NMDA. However, there was no significant difference in median seizure intensity or FJB staining between COX-2+/+ and COX-2-/- mice exposed to lindane. Associated with the increased susceptibility of COX-2-/- mice to NMDA and KA excitotoxicity was an alteration in GABAergic neurotransmission. In whole cell recordings of CA1 pyramidal neurons from hippocampal slices prepared from COX-2-/- mice, we observed a 60 % decrease in the frequency of spontaneous inhibitory postsynaptic currents (sIPSC). This alteration in GABA sIPSC frequency suggests that decreased GABAergic inhibition exists at CA1 synapses in COX-2-/- mice which may contribute to increased susceptibility to excitotoxins, such as KA and NMDA, that directly activate glutamatergic neurotransmission but not toxicants that indirectly alter glutamatergic neurotransmission, such as lindane. These results suggest that COX-2 activity modulates excitotoxin-induced seizures by decreasing GABAergic neurotransmission. [Supported by IRP/NIA/NIH and NIH grant MH64797 to SV].

1816 PROTEOMIC PROFILING OF PROXIMAL MURINE GIANT AXONOPATHY.

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Axonal degeneration is common to many neurological diseases but the pathogenetic mechanisms are unknown. 1,2-Diacetylbenzene (1,2-DAB), the neurotoxic γ-diketone metabolite of the aromatic solvent 1,2-diethylbenzene, reacts with neuroproteins and induces proximal giant axonopathy in spinal motor and other neurons. In contrast, its isomer 1,3-DAB does not react with proteins and fails to induce axonopahy. We exploited the protein-reactive and axonopathic properties of 1,2-DAB, and the non-protein reactive and non-axonopathic properties of 1,3-DAB, to identify protein targets of 1,2-DAB and elucidate mechanisms underlying axonal degeneration. We used two-dimensional differential in-gel electrophoresis and matrix-assisted laser desorption/ionization time-of-flight/ tandem mass spectrometry to characterize proteomic changes associated with giant proximal axonopathy in spinal cord of adult Sprague-Dawley rats treated intraperitoneally with 20 mg/kg/day 1,2-DAB, 5 days a week, for two weeks. Animals treated with equivalent doses of non-axonopathic 1,3-DAB, or vehicle (saline containing 2% acetone), served as controls. Our findings indicate that 1,2-DAB specifically altered the expression of several proteins, including those involved in maintaining the structural integrity of axons (e.g. gelsolin), in supporting energy metabolism (NADH dehydrogenase) and in responding to oxidative stress (glutathione S-transferase). The vulnerability of gelsolin, an actin capping and severing protein, may have particular relevance to 1,2-DAB-induced axonopathy. Supported by U19ES011384, K01NS052183 and the Oregon Worker Benefit Fund.

1817 EFFECT OF 1-BROMOPROPANE EXPOSURE ON GENE EXPRESSION OF NEUROTRANSMITTER RECEPTORS IN RAT.

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1-Bromopropane(1BP) is one of the widely used industrial solvents replacing ozone depleting solvents. Reproductive and nervous system are affected by 1BP in animal studies. Human cases were also reported in China and United States. Thirty six F344 rats were randomly divided into 4 groups of nine and exposed to 1BP at 0,400,800 and 1000ppm for 8 h/day, 7 d/wk for 4 wks. At the end of the experiment, rats were decapitated and the brain was dissected into the cortex, caudate putamen, midbrain, hippocampus, amygdale, pons-medulla oblongata and cerebellum. The brain parts were immediately frozen in liquid nitrogen and stored at -80 C degree. Total RNA from brain parts were extracted and reverse transcripted to cDNA. Real time PCR was done using cDNA for quantification of mRNA expression of serotonin, dopamine and GABA receptors. The cerebellum and caudate putamen did not show any significant change in mRNA expression of any receptors. The hppocampus showed clear dose-dependent decrease in 5HTr2a, D2R and GABAa1 by exposure to 1BP. The cortex showed significant decrease in 5HTr1a, 5HTr2a, D1R and GABAa1 at 800 ppm but did not extend this tendency at 1000 ppm. The amygdala showed increase in 5HTr3a at 400 and 100 ppm and in GABAg2 at 1000 ppm. The midbrain showed dose-dependent decrease in 5HTr3a. The pons-medulla showed decrase in 5HTr1a and 5HTr3a at 400 ppm or over. The cerebellum showed significant decrease in D1R only at 800 ppm. The caudate putamen did not show any significant changes. mRNA expression of D2R in hippocampus and 5HTR1a and 5HTR3a in the pons-medulla oblongata was the most sensitive indicator since they were suppressed at 400 ppm or over. The results suggest that mRNA expression analysis in dissected brain parts is useful in identification of the susceptible brain regions to 1BP exposure, as well as providing potential biomarkers for the evaluation of the central nervous toxicity.

1818 MEASURES OF MPTP BRAIN DOPAMINE NEUROTOXICITY.

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This study was initiated to further elaborate the brain effects of the neurotoxin MPTP (1-methyl-4-phenyl-1,2,5,6- tetrahydropyridine) over a period of years. Four female Macaca nemestrina laboratory monkeys received a unilateral intracarotid dose of MPTP. A modified method of Bankiewicz et al. (Life Sci. 39:7-16, 1986) was used to slowly infuse 2.5 to 3.5 mg of MPTP unilaterally into one common carotid artery of each adult monkey. After recovery from surgery, each animal was clinically rated on a parkinsonian scale, volitional responses to fruit presentation, scoring of significant hand and arm movements, and circling behavior. Positron emission tomography (PET) was used to quantify the pre- and post-synaptic consequences of dopaminergic neuronal damage. Three different radioligands were used to assess dopamine neuron loss in the lesioned compared to the intact hemisphere of each animal. MPTP induced dopamine neuron loss was assessed by: 1) [11C]dihydrotetrabenazine as a measure of the vesicular monoamine transporter VMATII loss. 2) Upregulation of postsynaptic dopamine D2 receptors by [11C]raclopride. 3) Loss of presynaptic $\alpha 4\beta 2$ nicotinic cholinergic receptors by 2[18F]fluoro-A-85380. The consequences of dopamine neuronal loss were primarily in the unilateral basal ganglia as visualized by quantitative PET imaging. (Supported in part by the University of Michigan Teaching and Research Development Fund 276157 and the Psychopharmacology Research Fund 361024.)

1819 VOODOO WASP TOXINS AND ZOMBIE COCKROACHES.

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The solitary parasitoid wasp *Ampulex compressa* uses a unique biochemical strategy to subdue its larger host, the cockroach *Periplaneta americana*. First, the wasp stings directly into the prothoracic ganglion of the cockroach, causing transient paralysis of its front (prothoracic) legs, which lasts for approximately three minutes. While the prothoracic legs are paralyzed, a second sting directly into the subesophageal ganglion (SEG) and brain cause dramatic behavioral changes. The cockroach immediately engages in pronounced grooming for approximately twenty minutes, and subsequently falls into a hypokinetic "zombie" state, lasting 1-2 weeks. The wasp then leads the compliant host into its burrow, whereupon it lays an egg on its victim, which serves as a food source for the developing hatchling. An adult wasp emerges from the hollowed-out cockroach approximately six weeks later. The mechanism of action underlying hypokinesia induction is currently unknown, but circumstantial evidence implicates impaired aminergic signaling. Biogenic amine levels in head and thoracic ganglia were quantified in stung and unstung cockroaches using high-performance liquid chromatography (HPLC) with electrochemical detection. The results show no significant differences, indicating that hypokinesia is not the result of venom-induced depletion of dopamine or serotonin. We are now testing the hypothesis that a component in the venom modifies postsynaptic dopamine receptors using receptor-based screening. D2-like receptors from *Periplaneta americana* and *Drosophila melanogaster* are being cloned into an expression vector suitable for heterologous expression in Chinese hamster ovary (CHO) cells. Receptor activation will be monitored through use of a heterologous chimeric G-protein, which couples G-protein activation to calcium mobilization and activation of aequorin, resulting in luminescence.

1820 ACUTE BEHAVIORAL EFFECTS OF INHALED PERCHLOROETHYLENE IN RATS ARE DIRECTLY RELATED TO ITS CONCENTRATION IN THE BRAIN.

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Perchloroethylene (PCE) is a volatile organic compound (VOC), frequently used in dry cleaning processes, that is currently being assessed by EPA for its risk to human health. Many VOCs are acutely neurotoxic and have been shown to affect attentional processes in humans and animals. The current study sought to assess the effects of inhaled PCE on sustained attention in rats performing a visual signal detection task (SDT). Because physiological effects of PCE are similar to those of other commonly used VOCs (e.g., toluene and trichloroethylene (TCE)), which are known to impair attentional processes in rats, we hypothesized (1) that acute inhalation of PCE (0, 500, 1000, 1500 ppm) would disrupt performance of the SDT in rats and (2) that the effects would correlate with concentrations of PCE in the brain as estimated by a physiologically-based pharmacokinetic (PBPK) model. As predicted, PCE impaired performance of the signal detection task by reducing accuracy (500 -1500 ppm), elevating response times (1000 -1500 ppm) and reducing the number of trials completed (1500 ppm). These effects were consistent with impaired attention and were closely related to estimated concentrations of PCE in the brain. These results show that PCE is similar to other VOCs in its acute effects on attention, and that these effects can be predicted by the concentration of PCE in the brain at the time when the behavior is measured. This abstract does not necessarily reflect EPA policy.

1821 ACUTE INHALATION OF 2, 2, 4-TRIMETHYLPENTANE ALTERS VISUAL EVOKED POTENTIALS AND SIGNAL DETECTION BEHAVIOR OF RATS.

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The volatile organic compound 2,2,4-trimethylpentane (TMP, "isooctane") is a primary constituent of gasoline for which the current health effects data are insufficient to permit EPA to conduct a risk assessment. We evaluated potential neurological impairment from acute inhalation exposure to adult male Long-Evans rats using both electrophysiological and behavioral assessments. Rats (n=9-10/dose) were exposed to TMP vapors in concentrations of 0, 500, or 1000 ppm. Visual evoked potentials were recorded from electrodes previously implanted in visual cortex while the rats viewed modulating visual patterns (0.16 cpd, 60% contrast, 4.55 Hz appear/disappear). VEPs were recorded before exposure, every 10 min during a 60 min exposure session, and for an additional 60 min after exposure terminated. The spectral amplitude of the frequency-double component (F2) was significantly reduced by 500 or 1000 ppm TMP exposure. In behavioral assessment, rats (n=14) performed a signal-detection operant task while breathing 0, 500, 1500, 2000, or 2500 ppm TMP for 62 min. Slight reductions were observed in accuracy measures at the 2000 and 2500 ppm concentrations. Together these data demonstrate that TMP, like other volatile organic substances, impairs neurological function during acute inhalation exposure. This abstract does not reflect EPA policy.

1822 CIRCADIAN CONTROL OF THE RAPID DEVELOPMENT OF TOLERANCE OR HYPER-REACTIVITY TO ETHANOL INDUCED SLEEP TIME IN RATS.

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Circadian influences on many biological systems have been well documented. Included among the phenomena which exhibit circadian rhythms are responses to various drugs. In general, rhythmic variations in drug response have been attributed

to changes in absorption, distribution, bio-ransformation, excretion, and tissue sensitivity. The present investigation was designed to examine the circadian control of ethanol induced sleep time, and to ascertain the presence of chronotolerance, or hyper-reactivity to ethanol in rats. Sprague-Dawley rats maintained under controlled light of 12L:12D light-dark cycle (lights on at 07:00 hr) and temperature condition were used. Different groups of animals were injected intraperitoneally with 4 mg/kg of ethanol at 06:00, 10:00, 14:00, 18:00, 22:00, and at 02:00 hr for three consecutive days, and the duration of sleep was measured. Sleep time was measured from the time of loss of righting reflex until regain of righting reflex. The results obtained indicate that animals exhibited a circadian variation in their responsiveness to sleep time. Maximum induced sleep time occurred during the dark phase, specifically at 22:00 hr, while minimum sleep time occurred at 06:00 hr at the end of the dark phase after the first injection. The results show that tolerance developed to ethanol injection. Repeated injection of ethanol after 24 hours was accompanied by decrease in sleep time. It was interesting to note that repeated administration of ethanol resulted in tolerance development only if animals were treated during the dark phase. Animals injected with ethanol at 06:00 hr for three consecutive days manifested a hyper-reactivity response or increase in sensitivity to ethanol induced sleep time. The present results indicate that tolerance, or hyper-reactivity to ethanol induced sleep time is a circadian controlled phenomenon.

1823 TOLERANCE TO ANATOXIN-A AND NICOTINE ON OPERANT BEHAVIOR: A SEARCH FOR MECHANISMS.

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Anatoxin-a is a cyanobacterial toxin of concern to EPA as it can contaminate water supplies, and poison a wide variety of species by stimulating nicotinic receptors. Research has shown tolerance to the effects of (+)anatoxin-a and nicotine on operant behavior with weekly administration. This experiment investigated behavioral and pharmacological mechanisms in tolerance development. Male Long Evans rats were trained to respond for food under a multiple schedule requiring completion of a variable number of responses (variable-ratio 30) or a response after a variable amount of time (variable-interval 60-sec). Groups (n=8) received weekly injections (s.c.) of either saline, nicotine (0.6 mg/kg) or (+/-)anatoxin-a (0.45 mg/kg) pre- and post-session for 4 weeks. Group assignments were: (1) vehicle pre- and post-session; (2) vehicle pre- and post-session for 3 weeks then nicotine or anatoxin- pre-session in week 4; (3) nicotine or anatoxin-a pre-session and vehicle post-session; (4) vehicle pre-session and nicotine or anatoxin-a post-session for 3 weeks then nicotine or anatoxin-a pre-session in week 4. Tolerance developed with weekly pre-session nicotine and, to a lesser extent, anatoxin-a. Weekly post-session nicotine or anatoxin-a had no effect on subsequent performance. Giving nicotine pre-session to rats that had received it post-session produced no greater effect than that obtained in rats having gotten it pre-session, indicating nicotine tolerance was due to a pharmacological mechanism. Giving anatoxin-a pre-session to rats that had received it post-session produced a slightly greater effect than that obtained in rats having gotten it pre-session, indicating tolerance to anatoxin-a was behavioral in nature. Finally, when cross-tolerance was tested, anatoxin-a rats showed complete tolerance to nicotine, whereas nicotine rats showed no tolerance to anatoxin-a. These results further highlight differences in the mechanisms underlying the behavioral toxicity of anatoxin-a and nicotine.
This is an abstract of a proposed presentation; the information does not necessarily reflect Agency policy.

1824 USE OF AN ORGANOTYPIC CULTURE SYSTEM TO STUDY THE ACTIONS OF MICROGLIA IN THE PRESENCE OF NEURONS EXPRESSING MUTANT HUNTINGTIN PROTEIN FRAGMENTS.

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Although mutated huntingtin (mhtt) has normal stability and cytoplasmic localization, it is prone to misfolding and aggregation, eventually forming nuclear and cytoplasmic inclusions. The presence of N-terminal mhtt fragments within neuronal processes precedes the appearance of nuclear inclusions and cellular degeneration. In the striatum of patients with Huntington's disease (HD), microglia increase in number and activation state in concert with increased GFAP expression, inclusion formation, and gradual neuronal loss. The interactions between microglia and HD-afflicted neurons are of interest for potential therapeutic targets, as microglial function is prone to pharmacological manipulation. Organotypic slice cultures (OSCs) provide a readily accessible platform that recapitulates the existing architecture of the brain in an intact tissue slice, replete with the myriad cell types present in vivo. Biolistic transformation of these cultures results in neuronal gene expression without ancillary trauma or noticeable activation of glia. We exploited this system to look at the effects of mhtt expression in striatal neurons (SNs) on the proximity and morphology of neighboring microglia during the timecourse of neurodegeneration. Expression of an N-terminal fragment of human htt containing 73 glutamines (N90Q73) resulted in a 50% decrease in viability at 5 days. We examined the response of B4 isolectin-labeled microglia, including their contact relationship, with mhtt- transformed SNs as compared to control neurons. Microglia were analyzed for size, clustering, deramification, and spheroid formation. Staining of cryosections demonstrated a differential in cells in close proximity to mhtt-expressing SNs. Manipulation of this glial activity within a system modeling the intact architecture of the brain may prove useful in developing new therapies aimed at relieving aberrant glial actions in HD. Supported by NIH intramural research and the Cure Huntington's Disease Initiative.

1825 MECHANISM AND TREATMENT OF SULFIDE-INDUCED COMA: A RAT MODEL.

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Dimethyl sulfide (Me2S) is a less potent analogue of hydrogen sulfide (H2S), which induces coma (colloquially termed knockdown) in humans. Unlike H2S, which acts by hydrosulfide (HS-), Me2S also induces coma in Sprague Dawley rats, providing a convenient model for investigating sulphide toxicity. The incidence and duration of coma induced by intraperitoneal injection of Me2S was determined over a range of doses. Ataxia was followed by coma, salivation, and death but no characteristic post mortem changes were found and no seizures were observed. The ED50 for coma was 813 mg/kg; complete recovery occurred in about 20 minutes after the maximal dose that was not immediately lethal. The LD50 at 24 hours after treatment was 537 mg/kg, making Me2S about 40-fold less toxic than NaHS. Low doses of NaHS, 7.5 or 10.0 mg/kg, with a low dose (sub-coma) of Me2S, also induced coma. Pre-treatment with NaHS, 7.5 or 10.0 mg/kg lowered the ED50 for Me2S-induced coma; this synergistic effect for coma suggests that a similar mechanism exists for toxicity of these analogues. Nitrite, pyruvate and dithiothreitol had no significant effect on coma or lethality from Me2S, whether given at onset of coma or as pre-treatment. Bicarbonate reduced duration of coma by about 28% but did not affect lethality. Bicarbonate and glucose, administered together, reduced coma duration by 37% and 80%, if given at onset and as pre-treatment respectively, and also reduced lethality from NaHS by 75%. This suggests that sulfide toxicity may be related to glucose metabolism.

1826 VALIDATION OF NEW PHOTOBEAM ACTIVITY SYSTEM USING ACRYLAMIDE IN WISTAR RATS.

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A quantitative assessment of motor activity is required as part of the functional observation battery (FOB) to detect potential neurotoxic effects. The purpose of this study was to evaluate the Photobeam Activity System (San Diego Instruments, USA) as part of the FOB conducted at JRF.
Acrylamide, a potent neurotoxic agent, was administered by oral gavage to groups of 5 male and 5 female Wistar rats at 15 and 30 mg/kg body weight, daily for 28 days. A control group was given normal saline solution. A full FOB assessment was conducted on Days 0, 7, 14 and 28. The assessment of motor activity was conducted using a Photobeam Activity System, (San Diego Instruments, USA) with an automated program to assess and analyze the data generated by the control and treated animals.
Acrylamide (30 mg/kg/day) caused a significant reduction ($p< 0.05$) in fine activity of males on Day 14 and of ambulatory activity of males and females on Day 28, compared to the control group. The total activity of females given 30 mg/kg/day was significantly reduced ($p< 0.05$) on Day 28. In addition, other measurements conducted as part of the FOB found neurotoxic effects. At 30 mg/kg/day the forelimb and hindlimb grip strength of both sexes was decreased on Day 28. The hindlimb foot splay of both sexes for 30 mg/kg/day was significantly increased ($p< 0.05$) on Day 28.
The above findings are consistent with the neurotoxic actions of Acrylamide reported in the literature. The FOB is an established approach to the identification of neurotoxicological effects and is a requirement of some regulatory guidelines for the testing of chemical products (OECD Guidelines 407 & 408, EPA Health Effects Test Guideline OPPTS 870.3100). In this study, use of the photobeam activity system allowed a quantitative assessment of motor activity as part of the FOB.

1827 SNC-34 CELLS AS A MODEL OF MOTORNEURON EXCITOTOXICITY.

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Spinal motorneurons are key components of the motor unit, and responsible for controlling neuromuscular function. Their function is impaired relatively selectively in humans with amyotrophic lateral sclerosis- ALS. The specific mechanisms by which motorneurons are damaged in ALS is poorly understood. We are testing the hypothesis that exposure to environmental metals facilitates development of ALS. Because of the difficulty of obtaining sufficient quantities of spinal motorneurons, having models in which motorneurons' physiological function can be studied, can provide an important tool to further these studies. NSC-34 cells, derived from a cross of neuroblastoma and spinal cord neurons, have been proposed for use as a model for motorneurons. The objective of this study was to begin characterizing Ca_i regulation and glutamate-mediated excitotoxicity in NSC-34 cells. Single cell microfluorimetry using fura-2 as an indicator for Ca_i was used along with the glutamate receptor agonist kainic acid to examine Ca_i. A "live-dead" cytotoxicity assay was used to assess glutamate-mediated cell death. Antagonists of ionotropic kainate/AMPA receptors (CNQX) and NMDA receptors (MK801, APV) were used to determine receptor subtype associated with the excitotoxicity. Kainic acid caused a concentration-dependent cell death. The number of cells affected by excitotoxicity was reduced by CNQX. Kainate also reversibly increased fura-2 mediated fluorescence. A subsequent application of kainate was variably effective in elevating fura fluorescence, indicating desensitization of the glutamate receptors. CNQX, but not APV or MK801 reduced significantly kainate-induced fura fluorescence. Thus NSC-34 cells express kainate/AMPA receptors, which cause significant elevations in Ca_i and subsequent cell death. These cells appear to be a suitable model for spinal motorneurons with which to study actions of environmental neurotoxicants on motor function. Supported by NIH grant R21-ES014357.

1828 ESTROGEN INCREASES TRANSTHYRETIN PRODUCTION IN, BUT NOT SECRETION FROM, THE CHOROID PLEXUS.

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Women who undergo estrogen replacement therapy (ERT) closely following menopause have a reduced risk of Alzheimer's disease (AD), yet the mechanism of this brain protection remains elusive. Estrogen has been shown to increase the gene expression of transthyretin (TTR), a protein that binds beta-amyloid (Aβ) peptides and prevents the formation of the toxic Aβ plaques. TTR is produced in the brain exclusively by the choroid plexus tissues, which secrete TTR into the cerebrospinal fluid (CSF). Recent work has identified the choroid plexus, the primary site of the blood-CSF barrier, as having a role in Aβ removal from brain. Our working hypothesis is that ERT reduces AD risk by increasing the production and secretion of TTR by the choroid plexus, which increases the formation of TTR-Aβ complexes and reduces the formation of Aβ plaques. To test this hypothesis, we determined the concentrations of TTR in tissue and fluid samples drawn from estrogen-depleted, normal, and estrogen-restored rats. Twenty-six female Sprague-Dawley rats (approximately 200 g) were ovariectomized and fourteen received sham surgery, leaving the ovary intact. One week after surgery, rats were divided into groups to receive either 17β-estradiol or vehicle by i.p. injection every 48 hours for 2 or 5 weeks. At the end of the treatment period, TTR content in collected tissue samples was determined by ELISA. ERT significantly increased the level of TTR in choroid plexus tissue but not in CSF, suggesting an increase in TTR production but not secretion. Furthermore, estrogen status did not alter TTR content in whole brain homogenate, serum, or liver samples. Taken with our previous finding that the choroid plexus accumulates Aβ peptides from CSF, this suggests that ERT may increase formation of TTR-Aβ complexes inside the choroid plexus, thereby reducing Aβ aggregation and plaque formation in the brain extracellular space.

1829 STANDARDISATION OF A QUANTITATIVE METHOD TO ASSESS NEURITE ELONGATION IN CULTURED RAT DORSAL ROOT GANGLIA (DRG).

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Certain esterase inhibitors cause clinical and morphological exacerbation (promotion) of toxic or traumatic axonal lesions. We already reported that effects on the formation of neurites of cultured rat DRGs caused by esterase inhibitors can be a model for studying promotion. However, data we presented so far on the effects of such compounds were mostly based on qualitative observations. Here we report on the standardisation of a quantitative method to assess the effect on neurite elongation. Desheathed DRGs from 13-16 day old rats were dissected and plated on poly-L-lysine pre-coated dishes. At 48 and 168 h, a picture (10x) was taken centered on the DRG, the field was divided in four quadrants and the numbers of neurites near the DRG (count A) and at the edge of the picture (count B) were blindly counted in each quadrant by four different observers. The elongation of the processes was assessed as the ratio between the number of neurites proximal to the DRG and the number counted distally (ratio A/B). When neurites were more than 60, they could not be precisely counted and the quadrant was defined as uncountable (UC). At 48 h neurites could always be counted and the ratio A/B was always >1.63 (all observers). At 168 h, many quadrants were UC: e.g. at least 1 quadrant B and 1 quadrant A were UC in 8 and 6 out of 9 DRGs, respectively. In the remaining cases the ratio A/B could be calculated and was <1.82 (all observers). This indicates that at 48 h a significant proportion of the neurites had not yet reached the distal part of the field, whereas at 168 h >50%, and often >75% of the processes did. Therefore, this time-points seem adequate to assess deficits of neurite elongation. The A/B ratios (mean±SD) of the 8 DRGS observed at 48 h were 2.55±0.56, 3.16±0.97, 2.17±0.81, and 2.48±0.79 for the four observers, respectively. The coefficient of variation between observers of the A/B ratio at 48 h was 10-40% (median 23). On this basis, the same observer will be involved in the final evaluation.

1830 UNCOUPLING PROTEIN-2 UP-REGULATION ENHANCES MITOCHONDRIAL GLUTATHIONE DEPLETION IN CYANIDE-INDUCED CYTOTOXICITY.

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We have shown that Wy14,643, a selective peroxisome proliferator-activated receptor-α (PPARα) agonist, sensitizes mesencephalic cells to cyanide-induced cytotoxicity by up-regulating UCP-2 expression by increasing the level of oxidative stress. Mitochondrial glutathione (mtGSH) is a vital component of the cellular antioxidant defense and its depletion appears to be involved in the neurotoxic response to cyanide. In this study, the relationship of UCP-2 expression to mtGSH depletion in cyanide toxicity was examined in the N27 mesencephalic cell line. Cyanide reduced both cytosolic GSH and mtGSH levels, and pretreatment with Wy14,643 enhanced the cyanide-induced decrease of mtGSH. Wy14,643-enhanced cyanide cytotoxicity was attenuated by restoration of mtGSH with either glutathione ethyl ester or N-acetylcysteine, further linking oxidative stress, mtGSH and possibly UCP-2 to the cytotoxic process. Knock-down of UCP-2 by RNA interference blocked both the Wy14,643 potentiation of cyanide-mediated reduction of mtGSH and the associated cell death, thus supporting a role for UCP-2. Using BSO (inhibitor of GSH synthesis) to further reduce GSH levels, it was observed that the level of UCP-2 expression and respiratory proton leak were markedly increased by Wy14,643. This was accompanied by a further reduction of mtGSH as well as cyanide-induced cell death. It was concluded that oxidative stress-mediated induction of UCP-2 expression decreases mtGSH to potentiate the cyanide-induced cell damage. (Supported by NIH grant ES04140).

1831 NEUROTOXICITY AND NEPHROTOXICITY OF AMPHOTERICIN MONO-METHYL ESTER.

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Amphotericin monoester (AME) is a polyene macrolide antibiotic highly effective against systemic infections by pathogenic species such as Coccidioidomycosis immitis. Early studies had shown that AME had less renal toxicity than its parent Amphotericin B (AMB), but its clinical use was terminated following reports of toxicity to CNS myelin. Recent studies using pure AME suggest that its neurotoxicity results from contamination by AMB, which can enter the brain when the blood brain barrier is compromised. We hypothesize that AME at therapeutic doses is not toxic to myelin. AME (7mg/kg ip) was given to adult Balb/c mice for 167 days, after which mice were killed by aldehyde perfusion and their brains and kidneys examined by light- and electron microscopy. No evidence of myelin disruption or pallor, or gliosis were observed following AME treatment, and the kidneys were histologically normal. However, when mice were administered AMB (5 mg/kg, ip) for as little as 14 days, evidence of edema, myelin-splitting, and intramyelinic edema was noted. Degeneration of renal tubular epithelium, with sloughing of cells into the tubular lumen, was present in the proximal convoluted tubule. Data suggest that pure AME is not neurotoxic at clinically relevant concentration. Given its exceptional therapeutic profile and low renal toxicity, AME should be reconsidered for clinical use in patients with resistant mycosis.

1832 ASTOROGLIAL DYSREGULATION OF VEGF EXPRESSION AFTER LPS TREATMENT IN DJ-1 KNOCKOUTS.

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DJ-1 is localized ubiquitously in the brain and mutations in the DJ-1 gene are associated with early onset of Parkinson's disease (PD) likely due to the loss of DJ-1 protein. Our laboratory has attained a new DJ-1 mutant mouse, and we have demonstrated it expresses virtually no DJ-1 RNA or protein. Upon exposure of these mice to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a known experimental inducer of PD, microvascular damage was identified. To investigate possible neurovascular dysfunctions, we are assessing vascular endothelial growth factor (VEGF) expression. VEGF has been identified as a critical player in cancer, ischemia, and inflammation in our mutant mice. Moreover, in a rat model of PD, it is reported that the survival of dopaminergic neurons and fibers were promoted after single injection of a VEGF-expressing adeno-associated virus vector into the striatum. Taken together, we set the objective of the study as defining the interrelationship of DJ-1 with VEGF in PD. As astrocytes have a key role in regulating neurovasculature, we are utilizing a combination of genetic predisposition with chemical agent to induce astroglial insult in order to determine the molecular mechanisms underlying PD. Our preliminary data show a dysregulation of VEGF, microvascular damage and astrogliosis associated with chemical exposure in DJ-1 knockouts. These facts indicate a disruption of endothelial-glial interrelationship, which may underlie the development of PD.

1833 RELEASE OF ENDOGENOUS DOPAMINE NEUROTOXIN 3, 4-DIHYDROXYPHENYLACETALDEHYDE (DOPAL) IN METHAMPHETAMINE (METH)TREATED PC6-3 CELLS.

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Methamphetamine (METH) is highly abusive drug in the United States and Europe. METH has been associated with loss of multiple markers for dopaminergic terminals in the brain. Among other causes, oxidative stress,excitotoxicity and mitochondrial dysfunction appear to play a major role in the neurotoxicity produced by METH. Many studies have been done to understand METH mechanism of toxicity; however neurotoxicity remains incomplete. The present study will focus on whether METH treatment yields elevated concentration of an endogenous dopamine neurotoxin 3,4-dihydroxyphenylacetaldehyde (DOPAL) in PC6-3 cells. It has been shown that long use of METH increases release of reactive oxygen species and lipid peroxidation products: malondialdehyde and 4-hydroxy-2-nonenal. These aldehydes are inhibitors of mitochondrial aldehyde dehydrogenase (ALDH) converting 3,4-dihydroxyphenylacetaldehyde (DOPAL), a reactive and toxic product of DA by monoaminooxidase, into 3,4-dihydroxyphenylacetic acid (DOPAC). Thus, inhibition of ALDH can lead to accumulation of DOPAL and eventually can lead to cell death. In addition, METH-induced release of DA from synaptic vesicle may also yield elevated levels of DOPAL. Therefore, we hypothesize that METH treatment yields increased concentration of DOPAL. Long term goal is to determine role of increased DOPAL in METH neurotoxicity. In this study, we treated differentiated PC6-3 (6x10(4)) cells with different dose of METH (100, 500, 1000 μM) for 4 hr at 37o C. Reaction was stopped by 5% perchloric acid and concentration of DA metabolites was determined by HPLC. Results show that METH dose-dependently increases extracellular DOPAL and decrease DOPAC. Interestingly, extracellular DA was reduced in METH-treated cells in comparison with control. These results suggest that METH treatment yields elevated DOPAL concentration by indirect effect on ALDH activity. Moreover, METH treatment may also affect on DA level by blocking DA transporter or inhibiting DA biosynthesis.

1834 *IN VITRO* MITOCHONDRIAL PROTEIN CARBONYLATION IN DITNC1 CELLS EXPOSED TO 1, 3-DINITROBENZENE.

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The oxidative modification of proteins by reactive oxygen species (ROS) is implicated as an important factor in the mechanistic development of neurodegenerative disorders. Protein carbonylation, a marker of oxidative injury, is associated with pathology seen in various neurodegenerative disorders. Exposure to 1,3-dinitrobenzene (1,3-DNB) leads to the development of gliovascular lesions in specific brainstem nuclei. Previous research shows that increased oxidative stress and mitochondrial dysfunction are characteristics associated with 1,3-DNB-induced neurotoxicity. The purpose of this study was to show that 1,3-DNB-induced production of ROS leads to loss of mitochondrial membrane potential ($\Delta\Psi m$) through carbonylation of specific mitochondrial proteins in DITNC1 cells. Experiments using 1mM 1,3-DNB or vehicle control (0.25% DMSO) were conducted to measure time-dependent loss of $\Delta\Psi m$ and time-dependent induction of superoxide anion. Loss of $\Delta\Psi m$ was determined by measuring TMRM fluorescence. Induction of superoxide anion was determined by measuring the fluorescence of MitoSOX Red. Partial loss of TMRM fluorescence in cells was apparent at 5 hr with complete loss occurring by 24 hr and peak MitoSOX fluorescence occurring at 5 hr. Pre-incubation with 600μM deferoxamine inhibited loss of TMRM fluorescence until the exposure reached 24 hr. 2D gel electrophoresis and Oxyblot analysis were used to determine if 1,3-DNB exposure led to the formation of mitochondrial protein carbonyls. 1,3-DNB exposure led to marked protein carbonylation in as early as 45 min, though not in the 0.25% DMSO control. Pretreatment with deferoxamine reduced the observed protein carbonylation. Tandem MS/MS was performed on mitochondrial proteins from 1,3-DNB treated cells for further identification. The results suggest that production of oxidative stress and loss of $\Delta\Psi m$ is associated with the formation of mitochondrial protein carbonyls. Since deferoxamine protection does not completely prevent the formation of protein carbonyls, oxidative carbonylation may occur through another mechanism.

1835 PROTEIN REACTIVITY AND ISOLATION OF DOPAMINE OXIDATION PRODUCTS.

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Parkinson's disease is a progressive neurodegenerative disorder that affects over a million patients in the U.S. The disease pathology is known to involve dopaminergic neuronal death. While the neurotransmitter dopamine (DA) itself is neurotoxic, neuronal death may be due to the inherent toxicity of certain dopaminergic metabolites such as 3,4-dihydroxyphenylacetaldehyde (DOPAL). This aldehyde is several orders of magnitude more toxic than the parent amine. The ability of DA to auto-oxidize to an ortho-quinone is believed to contribute to its cytotoxicity due to resultant protein reactivity and oxidative stress through redox cycling, reactive oxygen species production, and thiol depletion. However, little is known about the ability of DOPAL to similarly undergo auto-oxidation to a reactive quinone. If confirmed, the formation of DOPAL-quinone species would help explain the high toxicity of this aldehyde metabolite. NaIO4 and Tyrosinase, which are known to induce DA-quinone formation, were investigated for their ability to oxidize DOPAL. Colorimetric and spectrophotometric evidence suggests that these methods induce oxidative rearrangement of DOPAL. Such species were found to be reactive toward glutathione and N-acetyl cysteine, which are good indicators of protein reactivity. Following oxidation of DOPAL to the suspected quinone species, it was then trapped with N-acetyl cysteine, analyzed by HPLC, and purified by solid phase extraction for NMR analysis. Such spectrometric and chromatographic methods were utilized for analyzing and characterizing the rearranged and adduct-formation products of oxidized DOPAL in order to eventually determine their biological and cellular relevance, and establish any potential involvement in Parkinson's disease.

1836 GENE EXPRESS PROFILING MULTIPLEX QUANTITATIVE PCR ON AMPLIFIED RNA.

J. B. Eells. *Department of Basic Sciences, Mississippi State University, Mississippi State, MS.* Sponsor: M. Ross.

The gene eXpress profiling (GeXP) multiplex quantitative PCR assay uses a novel PCR chemistry in combination with capillary electrophoresis to measure expression of up to 35 genes in a single reaction. Briefly, reverse gene specific primers (GSPs) all having the same reverse universal sequence are used in the reverse transcriptase reaction to incorporate this sequence into the template. The PCR step uses forward GSPs with a forward universal sequence, and reverse and forward universal primers to amplify the cDNA. Previous experiments have shown that GeXP can detect most genes with 5-10 ng of RNA. Laser capture microdissection (LCM) can isolate more and more homogenous populations of cells, however, yields only small amounts of RNA, typically pg to low ng quantities. Since GeXP is stand specific, a potential limitation is compatibility with amplified RNA which, in most assays, is amplified into the antisense strand. In the current study, a multiplex reaction of 25 genes was designed and optimized to measure dopamine neuron genes as these neurons are lost in Parkinson's disease and are susceptible to various environmental toxins. This multiplex was tested on RNA from whole brain and micropunches of the substantia nigra that was amplified using the WT-ovation Pico RNA amplification system (NuGen Technologies) and the RiboAmp RNA amplification kit (Arcturus). The WT-ovation technique amplifies RNA into the antisense strand and converts it to DNA. Using different amounts of amplified DNA, however, failed to maintain the linear amplification observed when GeXP is used on RNA. Next, GeXP was tested on amplified RNA from the RiboAmp kit with the modification of switching the

GSPs used in the reverse transcriptase reaction and the PCR. GeXP on amplified RNA produced results similar to those observed using GeXP on RNA that was not amplified. These studies demonstrate the compatibility of using GeXP to measure gene expression on amplified RNA which expands the sensitivity of the reaction so that small amounts of RNA, such as those obtained via LCM, can be utilized.

1837 NEAR-INFRARED LIGHT TREATMENT ATTENUATES THE TOXIC EFFECTS OF MPTP IN A RODENT MODEL OF PARKINSON'S DISEASE.

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A large body of evidence supports a central role for mitochondrial dysfunction in the pathogenesis of Parkinson's Disease (PD). PD patients express systemic defects in mitochondrial complex I activity and complex I inhibitors have been shown to produce PD. There are also inherited forms of PD that result from mutations in genes that code for α-synuclein. The underlying pathological feature in both genetic and environmentally induced forms of PD is mitochondrial dysfunction resulting in increased production of reactive oxygen species followed by subsequent cell death of dopaminergic neurons in the substantia nigra. Photobiomodulation by far-red to near-infrared (630 to 1000 nm) light has been shown to enhance mitochondrial energy production and promote cellular survival following a mitochondrial insult. The present studies were undertaken to test the hypothesis that near-infrared (NIR) light emitting diode (LED) treatment will attenuate the toxic effects of MPTP in an in vivo model of Parkinson's Disease. In vivo experiments were performed in C57BL/6J mice. Mice were pre-treated with 670 nm LED light (5 minute duration) for 3 days prior to MPTP injection twice daily for a total of 6 pretreatments. Mice received one bolus injection of MPTP (50 mg/kg). Control mice received an injection of saline. Behavioral parameters were measured at 0-12, 23-24, 47-48, 71-72. Mice were sacrificed 72 hrs post-injection. 670 nm LED treatment reversed the toxic effects of MPTP by increasing the total number of movements made, increasing the time spent moving, increasing the distance traveled, and increasing the velocity of movement in C57BL/6J mice.

1838 NEUROTOXICITY STUDY DESIGN: THE ESSENTIAL ELEMENT OF LOCATION.

R. C. Switzer and C. L. Franssen. *NeuroScience Associates, Inc., Knoxville, TN.* Sponsor: D. Miller.

Within any organ, individual anatomical elements are specifically and uniquely vulnerable to toxic agents. Each of the different elements of the brain (e.g., hippocampus, thalamus, cerebellum, etc.) has a unique profile with regards to toxicity and each element warrants consideration. The brain has an incredible amount of diversity and complexity. There are over 600 distinct cell populations within the brain. Each division of the brain has different cell types, connectivity, and functionality. Our understanding of the brain has been increasing exponentially but we still do not fully understand the comprehensive functions of each population or the interactions of all the populations. In this study, we examine the neurotoxic profiles identified for various compounds. We find that (1) cells impacted by a neurotoxic compound are rarely widespread and are more often in small and specific regions of the brain; (2) neurotoxins often affect just one or perhaps several distinct and possibly distant regions; (3) affected regions can be very small, but functionally significant; (4) the location of effects is unpredictable based on other pathologic and behavioral indicators and even between compounds that share similar chemical structures. Although our understanding of the brain is perhaps not as complete as with other organs, we do not know of any regions of non-importance. These findings highlight the importance of adopting a well-defined safety assessment approach that examines all elements of the brain. Based on our research, we find that 50-60 uniformly-spaced coronal sections provide a complete, comprehensive sample of the brain's diverse structures.

1839 DELAYED SYSTEMIC ADMINISTRATION OF A NEW-GENERATION FATTY ACID AMIDE HYDROLASE INHIBITOR IS NEUROPROTECTIVE AGAINST EXCITOTOXIC SEIZURES, FUNCTIONAL COMPROMISE, AND BRAIN DAMAGE.

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The cannabinoid system has been implicated in protection against several disease states including excitotoxic events such as seizures. As an alternative to the direct action of CB1 receptor agonists, we have shown that irreversible inhibition of fatty acid amide hydrolase (FAAH) results in cellular protection as well as improved behavioral performance in excitotoxic rats (Karanian et al., JPET 322:1059, 2007). Here, we used the second generation reversible FAAH inhibitor AM5206, which has good bioavailability and interacts with the active site serine residue with moderate potency for controlled FAAH modulation. The inhibitor was injected IP (0-8 mg/kg) into rats immediately following kainic acid (KA), resulting in a significant reduction in seizures throughout the 4-h rating period. When AM5206 administration was delayed 2 h, the third and fourth hour reduction in seizures was similar to when AM5206 was given immediately after KA. In functional studies, AM5206 administered either immediately, or 2-8 h post-KA produced a similar level of behavioral protection, attenuating the KA-induced deficits in balance and coordination assessed at 24-48 h. AM5206 injected 24 h post-KA also produced evidence of behavioral protection. Lastly, brain tissue was harvested and analyzed for synaptic integrity and cellular pathology. AM5206 administration at the different post-KA times protected against excitotoxic cytoskeletal damage, preserved pre- and postsynaptic markers to levels comparable to those in non-insulted rats, and prevented the loss of pyramidal neurons and corresponding pyknotic changes. This study demonstrates that the new-generation FAAH inhibitor provides marked protection against epileptiform-type brain damage and is effective across a wide post-insult therapeutic window. (support: DA0732)

1840 CHANGES IN mRNA EXPRESSION PROFILES IN RAT CORTEX AND STRIATUM FOLLOWING SUB-CHRONIC TOLUENE EXPOSURE.

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Toluene, a volatile organic compound (VOC) used in many commercial products, is a ubiquitous air pollutant and therefore of interest to many EPA regulatory programs. A primary concern for toluene and other VOC's is the potential for persistent neurotoxic effects from long term exposure. While the acute actions of these chemicals have been studied extensively, little is known about the possible mechanisms by which VOC's may cause long-term effects on the CNS. Thus, a genomic approach was used to identify possible molecular and genomic mechanisms of neurotoxic effects following sub-chronic toluene exposure. Long-Evans rats were exposed to toluene via inhalation (0, 10, 100, 1000 ppm, n = 5 for each dose), 6 hrs/day, 5 d/wk for 13 weeks. The day following the final exposure, total mRNA was extracted from the cerebral cortex (CTX) and striatum (STR) and hybridized to high density Rat 230A Affymetric arrays. Changes in mRNA expression levels were analyzed in each tissue using two-way ANOVA with a false discovery rate of 0.05 to assess differential gene expressions. Analysis showed the number of significant genes as follows: 6995, 215 and 154 for tissue, dose and tissue/dose interaction, respectively. Principle component analysis showed separation between the control and 1000 ppm group in CTX, and a threshold in STR where control and 10 ppm were distinguishable from 100 and 1000 ppm. Thus, sub-chronic toluene exposure caused tissue and dose-dependent changes in mRNA expression. The present results may provide information regarding mechanisms underlying sub-chronic toluene neurotoxicity. (This abstract does not necessarily reflect agency policy).

1841 EVALUATION OF MEMORY FUNCTION IN RATS: A COMPARISON OF WATER MAZE AND OBJECT RECOGNITION TASKS.

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Cognitive functions have to be investigated in different types of preclinical safety studies, such as reproduction, developmental neurotoxicity or juvenile toxicity studies. For this purpose several types of test are commonly used, such as conditioning paradigms, mazes and water mazes, object recognition tasks, etc. In the work presented here, we have evaluated the effects of the same pharmacological agent scopolamine in three different testing approaches to assess cognitive abilities: the Morris water maze, Cincinnati Water Maze, Morris Water Maze and the Object Recognition Task. The first two methods test cognitive ability under aversive conditions, while the third method is based on the natural exploratory behaviour of rats. In the Morris Water Maze, scopolamine treated rats had longer swimming times when compared to controls, and the number of failures was markedly greater than controls on each day of testing. In the Cincinnati Water Maze, the administration of scopolamine did not significantly decrease performance during the learning trials when compared to controls. During memorisation trials, escape time and number of errors were higher in scopolamine treated rats. Effects on cognitive function after treatment with scopolamine were clearly demonstrated during the reverse phase of the test. In the Object Recognition Task at 2 hours after the training phase,

scopolamine treated rats showed reduced DI, indicating that scopolamine treated rats reacted to the new and familiar objects in the same way. In all three tests a sex difference was noted, females showing a greater sensitivity to the action of scopolamine than males.

1842 MITOCHONDRIAL RESPIRATORY INHIBITORS SENSITIZE ORGANOTYPIC HIPPOCAMPAL SLICE CULTURES TO EXCITOTOXICITY-INDUCED CELL DEATH.

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The broad-spectrum insecticide rotenone, an inhibitor of complex I of the mitochondrial electron transport chain (ETC), gives rise to oxidative stress and bioenergetic failure. Pesticides including rotenone have been implicated in human neurodegenerative diseases including Parkinson's disease. Another intensively investigated hypothesis of neurodegenerative disease involves the toxic action of the excitatory neurotransmitter glutamate. We demonstrated increased cell death in organotypic rat hippocampal slices exposed to sub-lethal rotenone and NMDA or AMPA for 24h. The increased cell death was synergistic at low concentrations and additive at higher concentrations of excitotoxin and appeared to be neuron-specific. In this study, we investigated the effects of mitochondrial toxins that inhibit complexes II – IV of the ETC. Organotypic rat hippocampal slice cultures were exposed to the ETC inhibitors concomitantly with NMDA (10 μM) for 24h then assessed for cell death as determined by propidium iodide (PI) fluorescence. Additionally, organotypic hippocampal slice cultures from transgenic mice expressing enhanced yellow fluorescent protein (eYFP) in astrocytic mitochondria were exposed concomitantly to rotenone (100 nM) and NMDA (10 μM) to further investigate the cell-type specificity of the PI-positive fluorescence. Sub-lethal concentrations of ETC inhibitors alone had little effect on cell death, but was significantly increased ($p < 0.01$) when co-administered with NMDA. Cell death in the mouse slice cultures, as determined by PI fluorescence and confocal microscopy, was evident in the pyramidal neurons with no overlap in the eYFP-positive astrocytes. These results suggest that inhibition of electron flow within the ETC potentiates excitotoxicity and is consistent with the possibility of promoting neurodegeneration.

1843 DISTRIBUTION OF 14C-PARAQUAT IN BRAIN AND BODY TISSUES OF C57BL/6 MICE USING PBPK MODELING AND AUTORADIOGRAPHY TOWARD AN ANALYSIS OF PARKINSON'S DISEASE-PATTERNED NEURODEGENERATION.

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A mouse PBPK model for paraquat (PQ) was developed (Toxicologist 96, #796, 2006) to understand brain dosimetry following PQ exposure. The model simulated the blood and brain kinetics of PQ reasonably well following IP, oral, dermal, and intranasal exposure except that the initial peak of brain PQ was overestimated, and amount in the brain was underestimated after intranasal exposure. As a direct-acting toxicant, the most appropriate dosimeter of brain exposure to PQ is the area under the curve (AUC), which was a linear function of the IP dose. A human PBPK model was developed based on the mouse model and used to simulate human dermal and inhalation exposure using various workplace scenarios. Based on the AUC for PQ in the brain, the equivalent IP doses in mice that correspond to human dermal workplace exposures were 6 to 9 orders of magnitude lower than the 10 mg/kg IP dose used in studies associating PQ exposure with neuronal loss in the substantia nigra (SN). The distribution of PQ was confirmed with quantitative whole body autoradiography to compare IP and intranasal routes of administration (μg 14C-PQ/g is reported in 43 tissues, including 10 brain regions). Microautoradiography of the midbrain, including the SN, compared regional concentrations of PQ.
The PBPK model was used to develop a human-relevant dose in a study to examine Parkinson's Disease-patterned neurodegeneration after PQ exposure. A lifespan mouse study is currently underway in which animals were dosed with 3 levels of PQ (the high dose based on positive literature at 10 mg/kg, the midrange dose based on a UK government operator exposure model, and the low dose based on this PBPK model). Animals were treated daily from postnatal day 5-19, and/or twice weekly from 6.5-7.5 months of age. Preliminary results indicate that there is no cross-transfer of PQ amongst pups within the same litter. In adults, the high dose was lethal to adult-only treatment males but not to females.

1844 METHYLMERCURY (MEHG) TOXICITY IN POSTNATAL RAT HIPPOCAMPUS IS ASSOCIATED WITH ACTIVATION OF THE MITOCHONDRIAL APOPTOTIC PATHWAY.

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MeHg insult during development induces a spectrum of harmful effects on brain structure and function. Recent studies have shown that a single injection of MeHg in 7 day old (P7) rat pups acutely activates caspase3, an apoptotic protease, leading to cell cycle arrest and cell death specifically in the hippocampus. Two major apoptotic pathways are known to control caspase3 activation: the mitochondrial pathway requiring caspase9 and the extrinsic pathway involving caspase8. Western analysis of P7 hippocampal extracts after sc injection of 5ug/g MeHg or vehicle revealed that both caspase9 and 8 were activated. However, caspase9 was activated at 2h and increased to 56% above control at 24h, while caspase8 was activated far less and at later time points. Analysis of cytochrome c release from the mitochondrial fraction, a process necessary for caspase9 activation, revealed a 25% release at 2h that increased to 40% above control by 6h. Finally expression of bax, a protein known to control cytochrome c release, increased by 24-38% above control between 2 and 4h after MeHg treatment. To validate the sequence of caspase activation, their activities were studied using caspase9 and 8 inhibitors (80uM Z-LEHD-FMK and 60uM Z-IETD-FMK, respectively) on E14.5 cortical cells with fluorescent substrates of caspase3, 8, or 9 after 3h treatment of 1.5uM MeHg. Results showed all caspase activity decreased by 80% when z-LEHD was added to MeHg treated cells, while z-IETD inhibited 25% of caspase3 and 66.7% of caspase8 activity when compared to the activity in cells treated with MeHg alone. These data suggest that MeHg toxicity involves activation of caspase3 primarily through the mitochondrial pathway. Starting with overexpression of bax, cytochrome c is released from the mitochondria activating caspase9, which in turn activates caspase8 and 3. Support: NIEHS: ES07148, ES05022, ES11256; USEPA R829391; FRM SPE2006.

1845 METHYLMERCURY INDUCES ALZHEIMER'S DISEASE-LIKE TAU PHOSPHORYLATION IN MOUSE BRAIN.

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Tau is an important cytoskeleton protein, and its hyperphosphorylation with increasing age is thought to be involved in neuronal cell death via the inhibition of microtubule polymerization in some neurodegenerative diseases such as Alzheimer's disease and frontotemporal dementia etc. However, since there is a difference at the progress speed in the individual level of the above-mentioned diseases, the pathogenic/progress factors were considered to be present in nature. Environmental toxicants are also thought to be the candidates as pathogenic/progress factors in some neurodegenerative diseases, and some researches have done about aluminum, arsenite and inorganic mercury which don't have the ability of brain penetration well. Methylmercury, an environmental neurotoxicant, has ability to pass through the blood-brain barrier and induces neuronal cell death. To understand the possibility of methylmercury as a pathogenic/progress factor in neurodegeneration disease, we used an in vivo model system using mice to investigate whether the Tau hyperphosphorylation is involved in the neuronal cell death induced by methylmercury. Mice were administrated methylmercury (30 ppm in free drinking water) for one to eight weeks, and detected in Tau protein and pathological changes in the brain. A noteworthy finding was that the mice exposed to methylmercury for eight weeks exhibited Tau hyperphosphorylation which is Alzheimer's disease-like Tau phosphorylation sites (Ser-199, Ser-202, Thr-205, Ser-262, Ser-404) in the brain. Furthermore, the neuronal cell loss and the TUNEL-positive neuron were observed in the same area. These results suggested that methylmercury might be a pathogenic/progress factor in some neurodegeneration diseases.

1846 ANALYSIS OF NEUROBEHAVIORAL DEVELOPMENT EFFECTS ON MICE AFTER TREATMENT WITH METHYLMERCURY DURING POSTNATAL BRAIN GROWTH SPURT.

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PURPOSE: The present study was designed to examine the long-term effects on neurobehavioral development after exposure to MeHg during postnatal brain growth spurt period. METHODS: Eighty ICR mice were given daily intraperitoneally injection of 0, 0.1, 1 or 3 mg/kg MeHg chloride randomly from PD15-17

and then tested at PD45 in the Morris water maze. After finishing the test, the mice were sacrificed by cervical dislocation, and the levels of NMDA receptor subtypes (NR1, NR2A and NR2B) protein in the hippocampus were measured by Western blot analysis. RESULTS: Although all the mice significantly improved in performance over blocks in the water maze, MeHg exposure groups had longer latencies ($F=2.88$, $p<0.05$) and swim distance ($F=2.97$, $p<0.05$) to find the hidden platform compared to the control group during the 4 days of acquisition training, and presented in dose-effect relationship. However, there were no significantly differences in platform crossings when the hidden platform was moved. Besides, neither swim speed nor gender differences were detected among the entire test. Western blot analysis results showed significant increases in the levels of the NMDA receptor subtypes (NR1, NR2A and NR2B) protein in the hippocampus in MeHg exposure groups compared to those in the control group. A significant reduction in body weight gain before PND40 was noted in the 3mg/kg group compared to the control group ($p<0.01$), and the difference disappeared after PD40. No other evident indications of toxicity were observed. CONCLUSION: Overall, the current study found that MeHg exposure during postnatal brain growth spurt induces the learning deficits induced by low MeHg exposure is subtle and persistent, and our research suggests that the neurobehavioral abnormalities of MeHg-exposed mice might be ascribed to alteration of the gene expression of specific NMDA receptor subunits in the hippocampus. Besides, in our study, MeHg exposure can cause reversible inhibitory effects on body weight gain, indicating a slight adverse developmental effect.

1847 METHYLMERCURY INHIBITS NITRIC OXIDE SIGNAL TRANSDUCTION IN DEVELOPING MOUSE CEREBELLAR CELLS.

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Methylmercury (MeHg) is a developmental neurotoxicant in animals and humans. It has been proposed that effects on developing of central nervous system (CNS) result at least in part, from inhibition of neuronal migration, however little is known regarding the molecular mechanisms, especially at low doses. We showed that LPS stimulation of cerebellar cultures increases neuronal migration at 48 h, an effect that was inhibited by a non-cytotoxic concentration of MeHg (Sass et al 2001). It is known that nitric oxide ($NO^{\bullet}$) is involved in neuronal migration and its production in brain is modulated by cytokines through inducible nitric oxide synthase (iNOS). Mercury compounds are known to inhibit cytokine-induced $NO^{\bullet}$ production and expression of the iNOS gene in cells of the immune system. The aim of the present study was to determine the effect of MeHg on $NO^{\bullet}$ production, mRNA expression and protein levels of iNOS in cerebellar cells. We used organotypic cells obtained from the cerebellum of 5 day old CD1 pups to evaluate effects of MeHg (100nM) in the presence or absence of LPS (50ng/ml) because of the role of LPS in neuronal migration. LPS activation increased $NO^{\bullet}$ production, mRNA, and levels of iNOS at 48 h. Only in the presence of LPS, MeHg decreased $NO^{\bullet}$ production, mRNA, and protein levels,. These effects of MeHg in LPS-stimulated cultures were concentration-dependent and Ca^{2+}-independent. Overall, these results suggest that the inhibition of neuronal migration by MeHg could be partially mediated by effect on iNOS pathways. *Research supported by Conacyt-46297.*

1848 MERCURY MODIFIES LPS-INDUCED GENE EXPRESSION IN DEVELOPING MOUSE CEREBELLAR CELL CULTURES.

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Prenatal exposures to methylmercury (MeHg) in humans and both primate and rodent models are known to affect the development of CNS, through inhibition of neuronal migration. We have previously reported that *in vitro* MeHg (100nM) can inhibit the migration of cerebellar granule cells induced by lipopolysaccharide (LPS) stimulation. To elucidate potential molecular mechanism involved in this effect, we studied the interactions between LPS and MeHg at the level of gene expression. Organotypic cerebellar cultures were established using tissue obtained from neonatal 5 day old mice and exposed *in vitro* to vehicle, LPS (50 ng/ml), or to LPS and MeHg (100nM). These conditions induce neuronal migration (LPS only) and, in the presence of MeHg, inhibit LPS-induced neuronal migration. A microarray with ESTs for 38,228 mouse genes was utilized to examine differences in gene expression under LPS-stimulated conditions as compared to unstimulated conditions, and also to determine MeHg-induced modification of the LPS-associated expression profile. A >2-fold change in signal was considered to be meaningful. LPS stimulation affected expression of 150 genes; 125 genes were upregulated (including genes related to several chemokines and cytokines) and 25 genes were down-regulated (including GFAP and CD86 antigen). MeHg exposure affected expression of only four genes also responsive to LPS: septin 4, thrombospondin 2, Stat3, RAS-related C3 botulinum substrate 2. Addiotionally, MeHg altered expression of 10 genes whose expression was not modified by LPS: these included chemokine (CXC motif) ligand 5, coronin actin binding protein 1A, myelin basic protein, transthyretin, epithelial membrane protein 1, and interferon regulatory factor 1ST. These results suggest that a limited number of genes and pathways may be involved in the inhibition of LPS-driven neuronal migration and possibly in the effects of MeHg on early brain development. *Research supported by Conacyt-46297 and by Cure Autism Now Foundation.*

1849 METHYLMERCURY INCREASES N-METHYL-D-ASPARTATE RECEPTORS ON SH-SY 5Y NEUROBLASTOMA CELLS LEADING TO NEUROTOXICITY.

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Methylmercury (MeHg) is a known neurotoxin but the mechanism for low dose chronic toxicity is still not clear. N-methyl-D-aspartate receptors (NMDA-R) were found to be induced after exposure to MeHg in a mink model but its role on neurotoxicity is not known. The aims of this study were to investigate the expression and the functional roles of NMDA-R on the induction of cell death in SHS5-SY human neuroblastoma cell line after exposure to MeHg. NMDA-R were measured using a radiolabeled phencyclidine receptor ligand [3H] MK-801 and cell death was quantified using fluorogenic substrates specific for caspase-3 (DEVD-AFC) and lactate dehydrogenase (LDH) release. We found a significant increase of NMDA-R follow by the increase of caspase-3 activity after 4 hours exposure to MeHgCl (0.25 to 1μM). The necrotic cell death was found after 4 and 24 hours to MeHgCl (0.25 to 5μM). The NMDA receptor antagonists dizocilpine ((+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d] cyclohepten-5,10-iminemaleate [(+)-MK 801]) or memantine (1-amino-3,5-dimethyl-adamantane) (15μM) completely attenuated MeHgCl-mediated cells death by blocking NMDA-R, thus demonstrating the importance of NMDA-R in mercury neurotoxicity. Intracellular calcium chelator BAPTA-AM (1μM) partially attenuated the neurotoxicity effect of 1μM MeHgCl. These results suggest that increase expression of NMDA-R trigger the enhancement of intracellular calcium leading to neuronal cell death.

1850 METHYLMERCURY DECREASES ERK1/2, p38 AND TAK1 PHOSPHORYLATION IN DEVELOPING MOUSE CEREBELLAR CELLS.

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Developmental exposure to methylmercury (MeHg) induces a spectrum of neurological alterations including cognitive disturbance and sensory/motor deficit. Some of these effects have been associated with disruption of neuronal migration, particularly of granule cells in the cerebellum, but the mechanisms by which MeHg can interfere with neuronal migration are not fully known, especially at low doses. Different signaling pathways are involved on neuron motility and recent studies have demonstrated that extracellular signal-regulated kinases (ERK) and p38 participate in neuronal migration. *In vitro*, MeHg can block LPS-induced increases in granule cell movement, suggesting alterations of signaling pathways associated with LPS-activation of glial cells. We studied the effects of MeHg on the signaling molecules ERK1/2, p38 and TGFβ-activated kinase (TAK1). Organotypic cultures were obtained from the cerebellum of 5 day old CD1 pups, and after 5 days in MEM media, LPS-stimulated cells were exposed to 100nM MeHg, for 15 min or 48h. ERK1/2, p38 and TAK1 were measured by Western blot using specific antibodies against nonphosphorylated and phosphorylated forms. As was expected LPS-stimulation at 15 min significantly induced ERK, p38 and TAK1 phosphorylation but at 48h only ERK1/2 and TAK1 phosphorylation was present. Exposure to MeHg significantly decreased the ERK, p38 and TAK1 phosphorylation induced by LPS at 15 min. MeHg treatment, at 48h decreased ERK1/2 but not TAK1 phosphorylation in LPS-stimulated cultures. Overall, the study demonstrates that MeHg can interact with LPS signaling in cerebellar cells through an inhibition of ERK, p38 and TAK1 phosphorylation, three kinases relevant to the LPS-pathway. These kinases could represent important targets associated with the inhibitory effect of MeHg on neuronal migration during early brain development. *This work was supported by Conacyt-46297.*

1851 METHYLMERCURY DECREASES NF-kappaB NUCLEAR TRANSLOCATION IN CEREBELLAR ORGANOTYPIC CULTURES IN VITRO.

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Methylmercury (MeHg) is known to be an environmental neurotoxicant associated with a range of neurodevelopmental disorders in humans. Furthermore, epidemiological and experimental studies have clearly shown that the developing nervous system is particularly vulnerable to MeHg toxicity. Fetal exposures to low doses of MeHg (primarily via fish consumption by pregnant women) has been shown to increase cognitive disorders (e.g. memory, attention and language problems) in children and adolescents, but molecular targets associated with these effects at low doses, relevant to the environmental exposures of human populations, remain unclear. Nuclear factor kappaB (NF-kappaB) is a transcription factor with ubiquitous roles in inflammation and other immune responses, as well as in control of cell division and apoptosis. NF-kappaB is known to be a target of mercury compounds in immune cells. In the nervous system, there is evidence that the NF-kappaB pathway is involved in functions such as memory and learning processes. Thus, the objective of this study was to determine the effects of low dose exposures of developing cerebellar cells to MeHg specifically related to NF-kappaB activation through its nuclear translocation and inhibitor kappa B (I-kappaB) degradation. In response to LPS activation cytoplasmic I-kappaB in cerebellar cells was decreased and nuclear levels of NF-kappaB were increased. MeHg treatment reversed the effects of LPS activation, in that nuclear NF-kappaB levels were decreased and cytosolic and nuclear I-kappaB levels were increased. These results indicate that MeHg may affect NF-kappaB signaling pathways in cerebellar cells *in vitro*, and that this may explain in part the effects of MeHg on the developing central nervous system. *Research supported by Conacyt-46297*

1852 COMPARATIVE EFFECTS OF METHYLMERCURY AND TOLUENE ON SYNAPTIC TRANSMISSION IN VISUAL CORTEX SLICES OF RAT.

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Both methylmercury (MeHg), a potent environmental toxicant, and toluene, an aromatic volatile compound, have been shown to cause impairments of visual function following acute and chronic exposure. To characterize their effects on the central visual synaptic function, we examined MeHg or toluene-induced changes in synaptic transmission in freshly isolated visual cortex slices of rat using extracellular microelectrode and whole cell patch clamp recording techniques. At 5 – 100 μM, MeHg decreased the amplitudes of both tetrodotoxin (TTX)-sensitive compound action potentials and glutamatergic field excitatory postsynaptic potentials (fEPSPs) in a concentration- and time-dependent manner. At 0.1 – 10 mM, toluene also caused a concentration- and time-dependent suppression of amplitudes of fEPSPs. However, the TTX-sensitive component was less sensitive to toluene when compared to fEPSPs, suggest that glutamatergic synaptic responses are more sensitive to toluene than are compound action potentials. Consistently, in whole cell recordings of glutamatergic spontaneous excitatory (sEPSCs) and GABAergic inhibitory postsynaptic currents (sIPSCs) in the visual cortex slices, MeHg suppressed both sEPSCs and sIPSCs in a concentration- and time-dependent manner although sIPSCs appeared to be slightly more sensitive to block by MeHg than were sEPSCs. In contrast, toluene was much more effective at suppressing GABAergic responses than it did glutamatergic responses. These results suggest that both MeHg and toluene affect synaptic transmission in the visual cortex, but their selectivity and potencies on glutamatergic and GABAergic responses differ. These differential effects may be related to the different characteristics of visual functional deficits induced by MeHg or toluene. Supported by NIH grant 5R21ES013767-01A2 and MSU funding 06-HBRI-II-616.

1853 COMPARATIVE GENE EXPRESSION ANALYSIS IN C57 MOUSE EMBRYOS UNDERGOING NEURULATION EXPOSED TO CADMIUM AND METHYLMERCURY.

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Neurulation represents a critical period in embryonic development where aberrations due to genetic and/or environmental factors may underlie neurodevelopmental diseases (NDDs) such as neural tube defects (NTDs). In humans, debate remains concerning the potential health effects of cadmium (Cd) and methylmercury (MeHg), however, in rodent models, both of these metals are potent developmental toxicants. Previously, we observed Cd to alter expression of several genes involved in CNS development and cell cycle regulation in mouse embryos undergoing neurulation associated with growth retardation and perturbed neural tube closure. Likewise, we hypothesize MeHg to affect expression of genes related to CNS development and cell cycle as well as expression of genes previously identified to be associated with NDDs. We used the Codelink Uniset 20K mouse I array to evaluate for gene expression alterations in C57 mouse embryos, 12 and 24 hrs post-injection (GD8.0, ip) exposed to vehicle, 4mg/kg(BW) CdCl2, or 4mg/kg(BW) MeHgOH. We employed ANOVA ($p<0.01$) to determine significant associations between time and treatment. Additionally, we used DAVID and gene lists established by two extensive reviews (Juriloff and Haines, 2007; Wassink et al., 2004) to analyze the effect of Cd and MeHg on gene expression in genes identified to be involved in CNS development, cell cycle regulation, and genes linked with particular NDDs of interest (NTDs and autism). Like Cd, MeHg induced alterations in genes involved in CNS development and cell cycle regulation, including several common targets (Elavl4, Pard3b, Cdkn1a, Bmp2). In addition, our study suggests that MeHg and Cd commonly impact genes implicated in NTD formation (Sall1, Cyp26a1) and autism (Dlx1, Ube2h). In summary, we have identified MeHg-induced gene expression alterations in C57 mouse embryos undergoing neurulation and highlighted several common gene expression targets affected by both Cd and MeHg. National Institute of Environmental Health Sciences, 1 U10 ES 11387-01.

1854 TEMPORAL PATTERN OF INCREASED OXIDATIVE DNA DAMAGE IN MURINE FETAL BRAIN AFTER IN UTERO EXPOSURE TO METHYLMERCURY.

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Methylmercury (**MeHg**) causes neurodevelopmental deficits in infants, possibly via generation of free radical intermediates that enhance the formation of reactive oxygen species (**ROS**). ROS-mediated oxidative damage to cellular macromolecules like DNA has been implicated in embryopathies caused by several xenobiotics. Previous studies in our lab found that fetuses exposed to a maternal dose of 8 mg/kg MeHg chloride exhibited increased levels of oxidative DNA damage, measured by high-performance liquid chromatography with electrochemical detection as 8-oxo-2'-deoxyguanine (8-oxodG), compared to fetuses exposed to a lower dose of 4 mg/kg, or to phosphate-buffered saline vehicle. The study herein sought to determine the time of peak levels of DNA oxidation in fetal brain after maternal exposure to MeHg. Pregnant CD-1 dams were injected on gestational day 17 with either MeHg chloride (8 mg/kg ip) or an equal volume of phosphate-buffered saline vehicle, sacrificed 2, 4 or 6 hr post-injection, and the fetal brains were collected. There were no differences in litter size or fetal viability among the three groups. Oxidative DNA damage in the brain was quantified as the level of 8-oxodG, an abundant and potentially pathogenic oxidative DNA lesion. Fetuses from the 6-hr group that were exposed to MeHg exhibited increased levels of 8-oxodG compared to fetuses from the 2-hr group, or the vehicle control group ($p<0.05$). These results suggest that oxidative stress and possibly DNA oxidation may play a role in the mechanism of neurodevelopmental deficits caused by in utero exposure to MeHg. (Support: NIEHS grant # ES013848; CIHR)

1855 METHYLMERCURY NEUROTOXICITY: ANALYSIS IN NOVEL GENETIC MODELS AND PRIMARY CULTURES.

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Methylmercury (MeHg) exposure from occupational, environmental, and contaminated foods is a significant threat to public health. The toxin easily passes the blood brain barrier and can cause severe psychological and neurological problems. Although MeHg poisonings have been studied for decades, the molecular determinants involved in the cellular pathology are largely unknown. A significant hindrance in identifying the molecular basis of the toxicity is the high complexity of the vertebrate brain and lack of facile in vivo genetic models to determine and explore the mechanisms involved in the cellular dysfunction. The genetically tractable nematode C. elegans and the zebrafish D. rerio are powerful models to identify and characterize the cellular, developmental, and molecular components involved in MeHg toxicity. The genes and regulatory pathways in these organisms are highly conserved with humans, and homologues of proteins involved in metal and oxidative stress responses in vertebrates have been identified in both models. Our initial studies indicate that C. elegans are highly sensitive to MeHg exposure, and that very low concentrations confer cell death. We have instituted genome-wide microarray analyses of gene expression following exposure to MeHg, and our results indicate that genes involved in protein folding, cell growth, and oxidative stress play significant roles in the neurotoxicity. We are currently exploring the role these genes

and others play in the toxicity in vertebrate cell and primary neuron cultures. We will also describe our plans to build on our in vivo studies that include a novel genome-wide screen to identify mediators and suppressors of MeHg neurotoxicity.

1856 MATERNAL METHYLMERCURY FROM A WILD-CAUGHT WALLEYE DIET INDUCES DEVELOPMENTAL ABNORMALITIES IN ZEBRAFISH.

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Methylmercury (MeHg) exposure is known to affect the eveloping nervous system in vertebrates, including humans. The goal of this project is to identify neural and molecular biomarkers as predictors of MeHg exposure and deficits in specific neurobehavioral endpoints. Specifically, we are investigating the effects of sublethal MeHg exposure on developing zebrafish by alyzing early lifestage (ELS) toxicity, changes in global gene expression by microarray analysis and quantitative real-time PCR (QPCR), and neurobehavioral dysfunction. We also exposed zebrafish embryos to varying concentrations of MeHg via maternal diet using freeze-dried, wild-caught walleye fillets that were destined for human consumption. Following analysis of Hg content, fillets were grouped as low, medium and high Hg content representing 1.0, 2.0 and 3.5 ppm dry weight, respectively. Maternal walleye diet resulted in an increase in embryo Hg content and an increase in ELS toxicity. Neurobehavioral deficits were also apparent in startle reflex response to a visual stimulus. Gene expression analysis in exposed embryos was performed using an 8,000 element custom zebrafish cDNA microarray. Microarray results were validated using QPCR. A number of the early life stage zebrafish genes found to be dysregulated by maternal MeHg have functional annotations involving cell division, cell cycle progression, neurodevelopment, liver development, ion-binding, transcriptional regulation and endopeptidase activity. These results show that numerous molecular pathways involved in the growth and development of multiple organ systems are disrupted by maternal exposure to moderate levels of dietary MeHg, leading to dramatic developmental and behavioral effects in their developing offspring.

1857 NEUROCHEMICAL BIOMARKERS TO ASSESS THE RISKS OF MERCURY IN NATURE USING COMPARATIVE AND INTEGRATIVE APPROACHES.

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Neurochemical biomarkers allow sub-clinical neurotoxicity to be assessed under the hypothesis that changes in brain chemistry precede structural/functional damage to the nervous system. Fish-eating wildlife are excellent models to study neurochemical biomarkers because they accumulate persistent toxicants, such as mercury (Hg), to threatening levels and brain tissues can ethically be obtained from trapped animals. Results from comparative (multiple organisms, neurological pathways) and integrative (in vitro, whole animal, field work) experiments are in support of this hypothesis. In vitro studies on brain tissues isolated from wildlife (mink, river otters, common loons, bald eagles, ringed seals, polar bears) show that Hg inhibits a range of neurochemical receptors and enzymes, but differences exist across species and between chemical forms of Hg (organic vs. inorganic). Cross-sectional studies on trapped animals show that concentrations of brain Hg are correlated with changes in neurochemical receptor levels (muscarinic, dopamine-2, NMDA) and enzyme activities (cholinesterase, monoamine oxidase). Causal relationships are being established by consulting the neurotoxicology literature, and analyzing brain tissues from mink fed MeHg (0-2 ppm) for 3 months in the laboratory. In summary, these results show that ecologically relevant levels of Hg can impair neurochemical signaling pathways in wild populations, and that neurochemical biomarkers can warn of early damage to the nervous system.

1858 NEUROCHEMICAL EFFECTS OF METHYLMERCURY ON GLUTAMATE AND GABA RECEPTORS – FROM THE BENCH TO THE FIELD.

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Methylmercury (MeHg) impacts glutamate and GABA receptors. Though, little is known about neurochemical changes in whole-organisms following chronic exposures to environmental levels. To address this, we carried out a range of experiments (in vitro, laboratory exposure, field study) on American mink, a fish-eating mammal that responds to MeHg similarly to humans. In isolated mink brain tissues, inorganic Hg (IC50 = 2-21 μM) inhibited ligand ([3H]-MK801) binding to the glutamate-NMDA receptor but MeHg (IC50 > 320 μM) did not. In 20 wild-trapped mink, a significant correlation (r = -0.71) was calculated between brain concentrations of MeHg and NMDA receptor levels. This correlation was verified in the laboratory as NMDA receptors were 15-44% lower in brain regions (basal ganglia, brain stem, cerebellum, occipital cortex) of captive mink fed ecologically relevant concentrations of MeHg (0.1 to 2 ppm) for three months. For the GABA(A) receptor, Hg did not inhibit ligand ([3H]-muscimol) binding in vitro. In wild mink, no correlations were measured between brain Hg and GABA(A) receptor levels. However, in laboratory-exposed mink, striking decreases in GABA-A receptor levels were found in several brain regions (92% loss in brain stem, 73% in basal ganglia, 42% in cerebellum, no effect in occipital cortex). By carrying out investigations at multiple tiers of biological organization, the results show that ecologically relevant levels of Hg (as low as 0.1 ppm in the diet) affect glutamate-GABA signaling in whole animals. Given that glutamate and GABA are the primary excitatory and inhibitory neurotransmitters in the brain, respectively, the mechanisms and implications of these findings will be discussed.

1859 DENTAL MERCURY EXPOSURE, 5-HTTLTR STATUS, AND ASSOCIATIONS WITH SELF-REPORTED SYMPTOMS.

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The effects of polymorphisms in the promoter region of the serotonin transporter gene(5-HTTLPR)have been reported to alter symptoms which are also affected by exposure to elemental mercury in humans. This study evaluated whether interactions exist between recent and chronic mercury exposure, the polymorphism, and current, recent(<3 months), and chronic(>1 year)symptom groups.

Symptoms were obtained by computerized questionnaire from 158 male dentists (DT's) and 84 female dental assistants (DA's) occupationally exposed to elemental mercury. Symptoms were obtained on same day as a spot urinary mercury and buccal cell were collected. Work histories were used to compute a chronic index of mercury exposure. Genders were analyzed separately. Scores for 11 symptom groups were regressed against current and chronic urinary mercury levels and genetic status for current, recent(<3 months), and chronic(>1 year) symptom time periods controlling for age, alcohol use, health problems, and use of medications.

Two selective adverse associations for confusion and anxiety symptom groups were observed with current and chronic mercury exposure across the 3 symptom periods for both genders(2 out of 11 groups at $p<0.05$). Adverse associations between the 5-HTTLPR mutant variant and recent symptom groups in males (5/11) and females (4/11) were more prevalent. Similar associations among chronic symptom groups were observed in females (5/11) and males (2/11). Across the three symptom time periods, there were 16 associations with the mutant versus 6 with the heterozygous status suggesting differencial sensitivity with the polymorphism. Our results found only two additive effects between mercury and the polymorphism for confusion and anxiety symptom groups indicating independent modes of action. These findings identified genetic determinants of specific self-reported symptomology that potentially could increase susceptibility to mercury toxicity in human subjects.

1860 MANGANESE EXPOSURE INDUCES ALZHEIMER'S–LIKE PATHOLOGY IN THE FRONTAL CORTEX OF NON-HUMAN PRIMATES.

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Exposure to high doses of manganese (Mn) is associated with cognitive, psychiatric and motor disorders in humans. This study is part of a multidisciplinary effort to characterize Mn-induced neurological dysfunction in non-human primates. One of the features of this model is overexpression of genes related to cell cycle regulation, apoptosis, inflammation, and in particular, upregulation of the gene encoding APLP1 protein belonging to the amyloid precursor protein family [Burton et al, Toxicologist 96, 1:19, 2007]. Histological examination of the frontal cortex in Cynomologous macaque exposed to $MnSO_4$ (10-15mg/kg, 10 months) revealed pathological features. Neurons of various types displayed swelling and intracytoplasmic vacuolization commonly observed in neurodegenerative disorders. In some cases, neuronal nuclei were depleted of nuclear staining and were hypertrophic similar to those described in Alzheimer disease (AD). Amyloid-β-immunopositive diffuse plaque-like aggregates were seen throughout the frontal cortex. Many neuronal and glial cells in the gray and white matter showed increased APLP1- and p53-la-

beling. Some of these immunopositive cells also had apoptotic phenotype suggesting an APLP1- and p53-dependent apoptotic response to elevated Mn concentrations in the brain. Accumulation of silver granules was detected in different neuronal types in both perykaria and neurites suggesting ongoing cellular neurodegeneration. Numerous Alzheimer type II astrocytes were also typical in the cortex of Mn-exposed animals. We frequently observed aggregates of hypertrophic protoplasmic astrocytes in gray matter and activated fibrous astrocytes throughout the white matter. These findings demonstrate that Mn-exposed non-human primates develop neurodegenerative changes resembling early AD pathology [Supported by NIEHS grant #ES010975 to TRG].

1861 NEUROIMAGING OF DOPAMINERGIC SYNAPSES IN THE STRIATUM OF MANGANESE-EXPOSED NON-HUMAN PRIMATES.

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Manganese (Mn) exposure produces a complex neurological syndrome with neuropsychological and motor function abnormalities. We have previously shown (Guilarte et al., Exp. Neurol. 202: 381-390, 2006) that *Cynomologous macaques* exposed to 3.3-5.0 mg Mn/kg (n=4) for approximately 10 months exhibit a marked decrease of amphetamine-induced dopamine release (AMPH-DAR) in the striatum measured by PET. No effect of Mn exposure on D2-dopamine receptors (D2R) or dopamine transporters (DAT) was observed. We now report results from PET studies of striatal dopaminergic synapses from additional animals (n=9) that were given doses of 5.0-6.67 and 8.33-10 mg Mn/kg in addition to those from the original study. Taken together (n=13), the present findings confirm that Mn exposure produces a marked decrease in striatal AMPH-DAR consistent with our previous findings. However, in animals that received higher doses of Mn, significant effects of Mn exposure on D2R and DAT levels in the striatum are also observed. Relative to baseline (prior to Mn administration), Mn exposure produces a significant decrease ($p<0.05$) in DAT at the first (Mn-1) and second (Mn-2) imaging time point after initiation of Mn administration. For D2R, a significant decrease relative to baseline was measured at the Mn-2 time point with a near significant decrease measured at Mn-1. No significant changes were measured from Mn-1 to Mn-2 for any of the PET measurements. These findings indicate that Mn affects both pre- and post-synaptic elements of the dopaminergic synapse in the non-human primate striatum. Based on the level of Mn exposure, AMPH-DAR is most sensitive to Mn exposure followed by DAT and D2R being the most resistant to Mn. [Supported by grant ES010975 to TRG]

1862 NEUROPATHOLOGICAL ANALYSIS OF GLIAL INFLAMMATORY RESPONSES TO MANGANESE-INDUCED NEUROTOXICITY IN DEVELOPING MICE.

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Chronic exposure to manganese (Mn) produces a neurodegenerative condition of the basal ganglia characterized by astrogliosis and activation of inducible nitric oxide synthase (NOS2). Induction of NOS2 causes overproduction of nitric oxide (NO) and injury to surrounding neurons, resulting in parkinsonian-like motor deficits. Inflammatory activation of astrocytes is believed to be an early event in Mn neurotoxicity but glial responses to Mn in developing animals remain poorly understood. In this study we investigated the effect of juvenile exposure to Mn in C57Bl/6 mice on the activation of astrocytes and microglia and production of nitric oxide, postulating that developmental Mn exposure would lead to selective increases in astrogliosis and increased expression of NOS2 with concomitant overproduction of NO. Mice were exposed to Mn by daily intragastric gavage. Immunohistochemical analysis revealed increases in expression of Glial Fibrillary Acidic Protein (GFAP), a marker of astrocyte activation, in the striatum, globus pallidus, and substantia nigra pars reticulata (snpr) of treated mice compared to controls. Additionally, the expression of the microglial maker, Iba-1, was moderately increased in treated mice. Co-immunofluorescence studies demonstrated increased expression of NOS2 primarily in astrocytes, as well in increase of 3-nitro-tyrosine protein adducts, a marker for NO/ONOO- formation, in neurons of the striatum, pallidum, and snpr of Mn-treated mice. In contrast, dopaminergic neuronal morphology remained unaltered in either the substantia nigra or striatum as indicated by immunohistochemical staining for tyrosine hydroxylase. These data indicate that sub-chronic exposure to Mn during development leads to inflammatory activation of astrocytes expressing NOS2, resulting in elevated nitrosylation of tyrosine residues in neurons of the basal ganglia.

1863 MANGANESE EXPOSURE ALTERS GABA AND NOREPINEPHRINE TRANSPORTER PROTEIN EXPRESSION IN THE DEVELOPING RAT BRAIN.

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Manganese (Mn) is an essential nutrient and cofactor for cellular processes, though exposure to high concentrations is known to result in neurotoxicity. Data suggest alterations in dopamine biology may drive the effects associated with Mn neurotoxicity, though recently other neurotransmitters have been implicated, such as γ-aminobutyric acid (GABA) and norepinephrine (NE). In addition, iron deficiency (ID), a common nutritional problem, may cause disturbances in neurochemistry by facilitating accumulation of Mn in the brain. Previous data from our lab have shown decreased brain tissue levels of GABA as well as decreased 3H-GABA and 3H-NE uptake in synaptosomes as a result of Mn exposure and ID. These results may be due to altered expression of transport proteins. Therefore, in this ongoing study the effects of Mn exposure and ID on the expression of proteins for GABA transporter (GAT-1) and NE transporter (NET) were examined. Weanling male Sprague-Dawley rats were randomly placed into one of four dietary treatment groups: control (CN; 35mg Fe/kg), iron-deficient (ID; 6mg Fe/kg), control with Mn supplementation (via the drinking water; 1g Mn/L) (CNMn), and ID with Mn supplementation (IDMn). After six weeks of dietary intervention, brains were dissected into globus pallidus, substantia nigra, and hippocampus, regions differentially affected by Mn exposure, and expression of GAT-1 and NET determined by Western blot analysis. In all three brain regions, Mn concentrations increased and Fe concentrations decreased in all treatment groups versus CN. Increased Mn caused approximately a 50% decrease in GAT-1 and NET protein expression overall, with the most dramatic results in the substantia nigra. In the substantia nigra, there was a decrease in expression of GAT-1 in CNMn (68%), ID (48%), and IDMn (44%) and a decrease in expression of NET in CNMn (72%), ID (22%) and IDMn (68%) versus CN. These data suggest that altered tissue levels of GABA and decreased uptake of GABA and NE due to Mn exposure may be the result of altered expression of their transport proteins.

1864 IRON CLEARANCE IN THE CEREBROSPINAL (CSF) OF RAT BRAINS AS AFFECTED BY MANGANESE EXPOSURE BY VENTRICULO-CISTERNAL BRAIN PERFUSION.

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Abnormally high levels of iron (Fe) in the brain have been associated with manganese (Mn)-induced neurodegenerative disorders. Our early studies suggest that Fe concentrations are elevated in the cerebrospinal fluid (CSF) of Mn-exposed rats. The choroid plexus (CP), where the blood-CSF barrier resides, may function to regulate Fe clearance from the CSF thereby maintaining Fe homeostasis in the brain. The current study was aimed at investigating whether the CP played a role in eliminating Fe in the CSF and how Mn exposure altered Fe clearance by the CP. Mn(II) as MnCl2 was administrated at a dose of 6 mg Mn/kg, i.p., 5 days a week for 4 consecutive weeks. At the end of treatment, rats were subjected to intraventricular insertion and cisternal cannulation. Following the infusion of [59Fe] and a space marker [14C]sucrose into brain ventricles, both [59Fe] and [14C]sucrose showed a time-dependent efflux in the cisternal CSF. [14C]Sucrose reached a plateau at 30 min after the initiation of perfusion, whereas [59Fe] reached the steady-state (plateau) at 50 min. In Mn-exposed group, the concentration of [59Fe] in the outflow reached the plateau more rapidly in Mn-treated animals (~20 min) than in controls (~30 min). [59Fe] recovered from the collected cisternal CSF was 25.5% greater in Mn-treated rats compared to controls ($p<0.001$), suggesting a reduced Fe uptake and/or removal by the CP. The outflow [59Fe] concentration corrected by [14C]sucrose in Mn-exposed rats was significantly higher than in control animals ($p<0.01$). The CSF formation rate (i.e., the secretion rate), as measured by use of the space marker [14C]sucrose, was not altered by subchronic Mn exposure. Thus, our data infer that an impaired uptake of Fe by the CP may contribute to a decreased Fe clearance from the CSF, which may in turn lead to an elevated Fe concentration in brain extracellular fluids seen in Mn-exposed animals.

1865 MANGANESE DISRUPTION OF MITOCHONDRIAL RESPIRATION IN THE BIVALVE *CRASSOSTREA VIRGINICA* AND ITS PROTECTION BY P-AMINOSALICYLIC ACID.

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Manganese is an essential metal that at excessive levels in the brain produces extrapyramidal symptoms called Manganism which is similar to Parkinsons Disease. The mechanism of action of manganese is not completely understood but is

thought to be due to factors including decreased brain dopamine levels, altered dopamine receptor activity and/or oxidative stress in mitochondria. p-Aminosalicylic acid (PAS) is a drug which recently is being shown to alleviate symptoms of Manganism. We studied the effects of manganese and PAS on mitochondrial respiration in gill of the bivalve mollusc, *Crassostrea virginica. C. virginica* gill is a tissue which is innervated by dopaminergic neurons. Mitochondrial respiration was measured using a YSI Micro-Biological Oxygen Monitor with a micro-batch chamber. Additions of manganese (0.1 - 10 mM) caused dose dependent decreases in mitochondrial O2 consumption. Adding PAS (0.1 - 1 mM) prior to manganese additions protected the mitochondria. The study demonstrates that manganese does adversely affect mitochondrial respiration and that the protective actions of PAS may in part be due to its ability to shield mitochondria from manganese induced oxidative stress. This work was supported in part by grants 2R25GM06003-05 of the Bridge Program of NIGMS, 0516041071 of NYSDOE, 0622197 of the DUE Program of NSF and 67876-0036 of PSC-CUNY.

1866 EFFECTS OF BLOCKING AGENTS ON MANGANESE AND CADMIUM ACCUMULATIONS IN GILL OF THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA.*

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Manganese is needed in small amounts for physiological functions. High amounts are toxic, causing Manganism. Welders and other metal workers subjected to high levels of manganese are targets for Manganism. It has been misdiagnosed as Parkinsons Disease because the two are similar and due to disruption in dopamine neurons. Cadmium, a trace metal with no known biological function in humans, adversely affects a various organs, including bone, kidney, liver and lung. Routes of exposure include cigarette smoke and ingestion of cadmium contaminated food. Cadmium pollution is widespread in aquatic environments. Marine animals, especially bivalves, accumulate it. Recently, the drug p-aminosalicylic acid (PAS) is being used to alleviate symptoms of Manganism, but its mechanism of action is unknown. The oyster *Crassostrea virginica* possesses a dopaminergic system innervating the gill. Work in our lab showed manganese disrupts this innervation. We now studied effects of PAS and EDTA on manganese and cadmium accumulations in oyster gill. Manganese and cadmium levels were measured using an Atomic Absorption spectrophotometer with a THGA graphite furnace. Gill from *C. virginica* was exposed to 0.5 mM manganese or cadmium for 10 hours, then treated with PAS or EDTA for three days. PAS treated gill, but not the EDTA treated, had lower accumulations of manganese compared to controls. PAS did not reduce cadmium accumulations, while high concentrations of EDTA effectively reduced the accumulations. This study is showing the mechanism of action of PAS for alleviating symptoms of Manganism may be partly due to reducing tissue accumulations of manganese. Cadmium accumulations cause serious toxic effects in animals and it can be valuable to find effective agents that remove cadmium from tissues and blood. This work was supported by the Louis Stoke Alliance for Minority Participation (LSAMP) in Science, and 2R25GM06003-05 of NIGMS, 0516041071 of NYSDOE, 0622197 of NSF, 0420359 of NSF and 67876-0036 of PSC-CUNY.

1867 ENHANCED PERIPHERAL AND CENTRAL INFLAMMATION IN MANGANESE EXPOSED MICE THAT WERE CHALLENGED WITH LPS.

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Manganism, caused by excessive exposure to the otherwise essential metal manganese (Mn), is a neurological disorder that resembles Parkinson's Disease (PD). While Mn has a direct effect on neuronal cells, several studies suggest that Mn also affects glial cells resulting in excessive inflammation. Recent data indicate that peripheral inflammation may play a role in neurodegenerative diseases. Hence, the objective of this study was to de-termine the potential interaction between Mn and a prototypical inflammogen, lipopolysaccharide (LPS) *in vivo.* C57BL/6 mice were exposed to Mn (3.5 mg/kg; *i.p.*) for 14 d with a subset being challenged with LPS (1.5 mg/kg; *i.p.*) on d 12. Behavioral analyses were performed at 4 and 48 h post-LPS administration and tissues collected at 4, 24, or 72 h post LPS. Brains were collected and processed for Mn, neurochemical, oxidative stress markers (F_2-isoprostanes, PGE_2), and western blot analyses. A significant brain accumulation of Mn occurred irrespective of LPS. Both Mn and LPS decreased locomotion, with the effect being greater for LPS. Importantly, the decrease in the open field distance traveled was greatest in mice exposed to both Mn and LPS. Neurotransmitter metabolism was increased in the striatum of mice challenged with LPS; the effect of LPS on certain metabolites was dampened by Mn. Both F_2-isoprostanes and PGE_2 levels were increased by LPS, an effect that was augmented by Mn. Importantly, mice exposed to both Mn and LPS, had markedly enhanced inflammatory response in both periphery and brain. Thus, peripheral inflammation may play an important role in the neurotoxicity of Mn. (Supported by NIEHS ES11654).

1868 ALZHEIMER'S DISEASE BIOMARKER, AMYLOID-BETA, IN THE BLOOD AND BRAIN OF DEVELOPMENTALLY LEAD EXPOSED MICE.

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Alzheimer's disease (AD) is a progressive neurodegenerative disease hallmarked by senile plaques composed of Amyloid-beta (Aβ) peptide aggregates. As definitive diagnosis can be achieved solely through post-mortem tissue, it is vital to evaluate whether Aβ in peripheral tissue can be correlated with Aβ concentrations in the brains of living patients. Our lab has previously shown that the neurotoxicant, lead (Pb), alters Amyloid Precursor Protein (APP) expression in the brain by inducing transcriptional up-regulation, thereby initiating an increase in Aβ. However, it is unknown if this alteration in the brain is also reflected in the blood. The objective of this study is to isolate Aβ in the blood and quantify its concentration in control and in Pb-exposed groups using a High Performance Liquid Chromatography (HPLC)-linked Enzyme-Linked Immunosorbent Assay (ELISA). Data analysis indicated that Aβ concentrations can be monitored through the assay and change in relation to control and Pb-exposure. These preliminary studies demonstrate that although Aβ levels are higher in the brain, it can be detected and quantified in the blood and can serve as an indicator of events in the brain.

1869 LIFETIME EXPOSURE TO LEAD AND STRESS: CONSEQUENCES FOR THE HPA AXIS AND BRAIN NEUROCHEMISTRY.

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Lifetime Pb exposure alone (0, 50 or 150 ppm in drinking water 2 mos before dam mating and continuing after weaning; no stress (NS)) or Pb+maternal stress (MS) or Pb+maternal stress+offspring stress at 4 mos (MS+OS) markedly increased Fixed Interval (FI) response rates in female but not male offspring in a Pb/stress-dependent fashion, i.e, in 50MS+OS, 150NS and 150MS+OS groups. To determine mechanisms of gender differences in behavior, levels of serum corticosterone, and of dopamine (DA), its metabolites DOPAC and HVA, and 5-HT and 5-HIAA in frontal cortex, nucleus accumbens and striatum were measured. At 2 mos of age, prior to behavioral testing or offspring stress, females showed Pb concentration-dependent increases in corticosterone levels to 74%; these increases were reversed by concurrent MS (Pb+MS groups). Similar trends in males were non-significant. Corticosterone changes were dynamic, however, with levels of all Pb alone and Pb+stress female groups significantly lower than controls after behavioral testing (10 mos), with the greatest reductions seen with 150 ppm Pb alone (72%) and with Pb+MS+OS (60-73%); males showed no reductions. Females exhibited altered levels of DA and 5HIAA in frontal cortex, HVA in nucleus accumbens and DOPAC in striatum, and changes in DA turnover in frontal cortex and nucleus accumbens, but neither these nor corticosterone changes could be straightforwardly related to the profile of FI rate changes. Pb+stress treated females differed from controls in the absence of an involvement of frontal cortex DA coupled with the inclusion of striatal and frontal cortex DA turnover as significant components explaining data in principal component analyses exploring relationships among outcome measures. Future experiments will determine mechanisms of Pb+stress-induced corticosterone changes and HPA axis dysfunction as well as profiles of neurochemical changes that underlie the associated increases in FI response rates. ES02ES012712.

1870 LEAD (Pb^{2+}) EXPOSURE ALTERS DEVELOPING GLUTAMATERGIC SYNAPSES IN CULTURED HIPPOCAMPAL NEURONS.

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Experimental evidence suggests that the ionotropic glutamate-type receptor N-methyl-d-aspartate (NMDAR) mediates Pb^{2+} effects on synaptic plasticity and cognitive function (Toscano and Guilarte. Brain Res Rev. 49:529-54, 2005). Maturation of glutamatergic synapses includes the recruitment and stabilization of NMDAR to dendritic spines apposed to pre-synaptic elements.

In this study, we investigated the effects of Pb^{2+} exposure (10-1000 nM) on pre-synaptic and post-synaptic markers in developing hippocampal neurons at 7 days *in vitro* (DIV7). To examine the effect of Pb^{2+} on developing synapses, we assessed the co-localization of NMDAR subunits with the pre-synaptic marker synaptophysin (SYN). Co-localization of NR1 and NR2A with SYN was significantly decreased by Pb^{2+} in a dose-dependent manner. No effect of Pb^{2+} on NR2B subunit co-localization with SYN was measured. These findings suggest that Pb^{2+} exposure decreases the development of NR1/NR2A synapses in hippocampal neurons with no effect on NR2B-containing synapses. We also measured the size and number of puncta containing post-synaptic density 95kDa (PSD-95) protein as a measure of dendritic spine development. At the lowest concentration of Pb^{2+} tested (10 nM), PSD-95 puncta area was significantly increased relative to the control condition suggesting a Pb^{2+} effect on dendritic spine size. No significant effect was measured on the number of puncta per μm. Western blotting of NMDAR subunits showed no effect of Pb^{2+} on NR2B subunit while NR1 and NR2A subunit protein levels were too low to accurately measure at this age and plating density. There was a significant effect ($p=0.037$) of Pb^{2+} on SYN protein levels with significant decreases occurring at 100 and 1000 nM. Further, Pb^{2+} exposure did not alter MAP2 protein levels indicating the lack of an effect on total neuron cell number. These findings provide supporting evidence that Pb^{2+} exposure alters the development of glutamatergic synapses in hippocampal neurons [Supported by grant ES06189 to TRG and NIEHS T32ES07141]

1871 HIPPOCAMPAL NEUROGENESIS AND BDNF FUNCTION ARE IMPAIRED BY CHRONIC LEAD EXPOSURE.

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The EPA must consider sensitive subpopulations in their assessment of health risk. Lead (Pb) is a developmental neurotoxicant that impairs hippocampal synaptic plasticity. Brain-derived neurotrophic factor (BDNF) has been implicated in structural and synaptic plasticity. The present study investigated the role of BDNF in Pb-induced neurotoxicity. Rats exposed from birth or weaning to 0, 0.1, or 0.2% Pb in drinking water were assessed as adults for dentate gyrus neurogenesis, hippocampal long-term potentiation (LTP), and stimulated release of BDNF. To assess neurogenesis, animals were administered bromodeoxyuridine (BrdU, 50 mg/kg, 2X daily for 12 days) to label newly born cells, sacrificed, and prepared for immunohistochemistry 1 and 28 days after the last injection. Continuous Pb exposure beginning at birth or weaning reduced the number of BrdU-positive cells at 28 days (-44%), indicating decreased cell survival. Hippocampal slices from these animals were preincubated with 40 ug/ml BDNF for 2 hr before application of LTP-inducing trains, a procedure that augments stimulation-induced LTP. BDNF-induced elevation in LTP was diminished in slices from Pb-exposed animals in both excitatory postsynaptic potential slope and amplitude. BDNF release was evaluated in hippocampal slices from a third set of control and Pb-exposed rats. Slices were allowed to equilibrate for >1 hr prior to transfer to a 96-well plate coated with a BDNF antibody. BDNF release induced by theta burst stimulation was measured via ELISA. Stimulated BDNF secretion was reduced in both the 0.1 (-28%) and 0.2% (-38%) Pb groups. These findings suggest that chronic Pb reduces BDNF secretion and TrkB receptor function, and may be a component of the diminished capacity for plasticity and neurogenesis in the adult hippocampus. (Supported by Children's Miracle Network. This abstract does not necessarily reflect EPA policy)

1872 IDENTIFICATION AND EVALUATION OF CHRONIC LOW-LEVEL LEAD TOXICITY BIOMARKERS IN NEONATAL RATS.

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The deleterious health effects of environmental lead(Pb) exposure have been observed and documented in numerous populations. Children, in particular, are very vulnerable to Pb toxicity. At the protein level, Pb substitutes for polyvalent cations, such as Ca2+. In proteins that bind Ca2+ for their function this Pb substitution can interfere with the function of the protein. The focus of our research is the evaluation of molecular biomarkers that can be used as a diagnostic of chronic low-level toxicity. In order to identify valid molecular markers we have focused on a more complex model system comprised of neonatal and maternal Long evans rats. Animals were divided into four treatment groups (n=4/group) with each group exposed to Pb acetate via drinking water ad libitum. After parturition the pups continued receiving Pb through lactating mothers. Pups were sacrificed on PND 35 and brains were dissected out for further biochemical analysis. Blood Pb levels in each pup were confirmed by the ICP atomic emission spectroscopy. Two treatment groups showed delayed parturition and a decreased number of pups as compared to untreated control. This effect was significant in higher concentrations of Pb. The pups receiving the highest concentration of Pb showed a decreased rate of development as compared to the control.

Previous toxicoproteomic work in our lab had identified the neuronal SNARE (Soluble NSF Attachment protein Receptor) proteins as molecular targets of low-level Pb intoxication. The neuronal SNARE proteins SNAP-25, Synaptobrevin, and Syntaxin are vital components of the vesicular neurotransmitter release machinery. Using our animal model system the SNAREs and associated proteins were evaluated. Protein expression patterns were characterized and evaluated using traditional western gel analysis. Gene expression patterns were characterized with RT-PCR and microarray analysis.

1873 CHRONIC LOW-LEVEL LEAD ACETATE (PBAC2) EXPOSURE DISRUPTS LOCOMOTOR ACTIVITY AND THYROID HORMONE LEVELS IN THE MALE ALBINO RAT (PRELIMINARY RESULTS).

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Lead (Pb) is one of the most common and pervasive environmental contaminants. It is well known that low level lead exposure disrupts brain neurochemistry leading to alterations in behavior such as control of movement, sensory and cognitive processing in human and in animal models. The present study reports the effects of chronic (five months) low level lead exposure on locomotor activity and the content of thyroid hormones in male Sprague-Dawley rats treated after weaning with 50 or 150 mg PbAc2/L of drinking water (d.w), while control group received 150 mg sodium acetate/L of d.w. As an outcome of this exposure protocol, PbAc2 exposed rats showed a significant increase in blood lead content. A steady decrease in the rats locomotor activity was found during the course of the experiment, which became statistically significant in the dark phase of the dark-light cycle after five months of PbAc2 exposure. Triiodothytonine (T3) content in the thyroid gland was increased in groups exposed to 50 or 150 mg PbAc2, while no changes in T3 were found in serum or liver. Although preliminary, these results shown that chronic low-level PbAc2 exposure causes an abnormal storage of T3 in the thyroid gland besides disrupting locomotor activity. More studies such as the assessment of deiodinase activity are needed to unravel the possible connection between chronic lead exposure and alterations on thyroid function and the consequent changes in locomotor activity patterns. Supported by PAPIIT-IN218307, PAPIIT-IN225305-3, CONACYT 46161-M.

1874 LOW-LEVEL HUMAN EQUIVALENT GESTATIONAL LEAD EXPOSURE PRODUCES GENDER-SPECIFIC MOTOR AND COORDINATION ABNORMALITIES AND LATE-ONSET OBESITY IN YEAR-OLD MICE.

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Low-level developmental lead exposure is linked to cognitive and neurological disorders in children. However, the long-term effects of gestational lead exposure (GLE) have received little attention. The goals of our experiments were to establish and validate a murine model of human equivalent GLE and to determine dose-response effects on maternal and litter measures, body weight, motor functions and dopamine neurochemistry in year-old offspring. Therefore, female C57BL/6 mice were exposed to water containing 0, 27 (low), 55 (moderate) or 109 ppm (high) lead from two weeks prior to mating, throughout gestation and until postnatal day 10 (PN10). Maternal and litter measures, blood lead concentrations ([BPb]) and body weights were obtained throughout the experiment. Locomotor behavior in the absence and presence of amphetamine, running wheel activity, rotarod test and dopamine utilization were examined in year-old mice. Peak [BPb] were <1, ≤10, 27 and 42 μg/dL in control, low-, moderate- and high-dose GLE groups, respectively. GLE had no effect on maternal or litter measures. Year-old male, but not female, GLE mice exhibited late-onset obesity. Similarly, we observed male-specific decreased spontaneous motor activity, increased amphetamine-induced motor activity and decreased rotarod performance in year-old GLE mice. Levels of dopamine and its major metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) were altered in year-old male mice, although only forebrain dopamine utilization increased. GLE-induced alterations were consistently larger in low-dose GLE mice. Our novel results show that GLE produced permanent male-specific deficits. The nonmonotonic dose-dependent responses showed that low-level GLE produced the most adverse effects. These data reinforce the idea that lifetime measures of dose-response toxicant exposure should be a component of the neurotoxic risk assessment process.

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1875 OXIDATIVE STRESS (OS) AND NEUROTOXICITY: ANTIBODIES IN RATS EXPOSED TO ORGANOMETAL ELECTROPHILES.

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Heavy metals impact the developing nervous system and may contribute to neurodegeneration in later life. Biomarkers of neurotoxicity to monitor risk and implement intervention strategies are needed. Male Fischer 344 rats (60 days) received trimethyltin (TMT), or trimethyl lead (TML) at 16 ppm in the drinking water and compared to water-only controls (n=8/group). ELISA for serum antibodies, IgM and IgG, against neurofilaments (NF), glial fibrillary acidic protein (GFAP) and myelin basic proteins (MBP) were used as biomarkers of neurotoxicity confirmed by measurement of brain GFAP, a marker of astrogliosis, in rats on day 12 of exposure. Antibodies against cardiolipin (CL) and oxidized LDL (oxLDL), in addition to brain thiobarbituric acid-reactive substances (TBARS), were used as measurements of OS. While sera from controls did not have detectable levels of antibodies against neural proteins, sera from rats exposed to either metal had both IgM and IgG against all neural antigens. IgM titers against NF-L and MBP were significantly ($p < 0.001$) higher with TML exposure, whereas IgG titers against NF and GFAP were more prevalent and significantly ($p < 0.001$) higher with TMT. This suggests that neurotoxicity was more advanced with TMT exposure. Furthermore, anti-GFAP, both IgM and IgG, were consistently higher in this group. TMT has been reported to be gliotoxic. Brain GFAP was significantly ($p < 0.001$) elevated in hippocampus of rats exposed to TMT or TML and in the cerebellum for those exposed to TML. This regional vulnerability is consistent with the neurotoxicity of these agents. OS was indicated by significant increases in TBARS in the hippocampus and cerebellum of rats exposed to either metal. Furthermore, anti-CL and anti-oxLDL were detected in sera of both groups, with oxLDL being significantly ($p<0.05$) higher in TML exposure. Despite receiving equivalent levels of oragnaometals and similarities as electrophiles that induce OS, other mechanisms underlie their differential neurotoxicity. This study supports the utility of antibody detection as a biomarker of neurotoxicity and OS.

1876 MODULATION OF CADMIUM-INDUCED OLFACTORY DAMAGE BY CALCIUM IN LARVAL ZEBRAFISH.

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We have previously shown that 96 h sublethal cadmium (Cd) exposures cause induction of the heat shock stress response, degeneration of the olfactory epithelium, and functional deficits in sensory perception in larval zebrafish. To determine if Cd was entering the cells by acting as a calcium (Ca) antagonist, zebrafish larvae (3 days old) were co-exposed to 1, 5, or 10 μM Cd and 1 or 5 mM Ca for 96 h. Induction of the heat shock response was visualized using a stable transgenic strain of *hsp70/eGFP* (enhanced green fluorescent protein) zebrafish. In the presence of an excess of Ca, the heat shock stress response in the olfactory epithelium was greatly reduced at all Cd exposures with 1 mM Ca and almost abolished with 5 mM Ca co-treatment, as only a few cells were expressing the reporter gene at 10 μM Cd with 5 mM Ca. Histological analysis revealed that the olfactory epithelium was less affected by Cd exposure when co-treated with either concentration of Ca, compared to Cd exposure alone. Also, TUNEL cell death assays indicated fewer cells were dying in the olfactory epithelium with a 5 mM Ca co-treatment with 5 or 10 μM Cd. Additionally, aversion assays using L-cysteine, an odorant known to cause aversion, were used to measure olfactory function. Fish exposed to 5 or 10 μM Cd had a decreased response to L-cys, indicating functional deficits. However, when co-treated with Ca a greater proportion of the Cd exposed larvae responded to the stimulus, suggesting that there was less Cd-induced damage to the olfactory system in the presence of Ca. Together, these results indicate that Cd gains entry to the olfactory epithelium via Ca uptake systems, wherein it causes damage to the olfactory system and sensory dysfunction.

1877 ACUTE SODIUM TUNGSTATE INHALATION IS NOT ASSOCIATED WITH DIRECT OLFACTORY TRANSPORT OF TUNGSTEN (188W) TO THE RAT BRAIN.

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Aerosol cloud formation may occur when certain tungsten (W) munitions strike hard targets. Pharmacokinetic mechanisms involved in the brain delivery of inhaled W are incompletely understood. The axonal transport of manganese, cadmium, and many other metals within the olfactory system represents one possible mechanism for direct delivery to the CNS. The objective was to determine whether inhaled W can likewise undergo direct olfactory uptake and transport to the rat brain. Male, 16-week old, CD rats underwent a single, 90-min, nose-only exposure to a Na2188WO4 aerosol (0.26 mg W/m3; MMAD 1.5 μm). Rats had the right nostril plugged to prevent nasal deposition of 188W on the occluded side. The left and right sides of the nose and brain, including the olfactory pathway and striatum, were sampled at 0, 1, 3, 7, and 21 days post-exposure. Gamma spectrometry was used to compare the levels of 188W found in blood on the left and right sides of the nose and brain to determine the contribution of olfactory uptake to brain 188W levels. Nasal respiratory and olfactory epithelial samples from the side with the occluded nostril had significantly lower end-of-exposure levels of 188W activity confirming the nasal occlusion procedure. Levels of 188W observed in all brain structures examined (olfactory bulb, olfactory tract/tubercle, striatum, cerebellum, rest of brain) paralleled 188W blood concentrations (at ~ 2-3% of blood levels), were highest at the end of the exposure, and returned to near back ground concentrations within 3 days. 188W concentrations in all brain structures taken from the right and left hemispheres were statistically equivalent suggesting that the olfactory route does not contribute to the 188W found in the olfactory pathway or other CNS structures.

1878 NICKEL (NI) NANOPARTICLE (NP)-INDUCED OXIDATIVE STRESS (OS) AND NEUROANTIBODIES.

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There is a clear need for a better understanding of the potential adverse health effects associated with nanotechnologies. Based on studies of ultrafine particles, NPs gain access to the CNS via axonal transport from the olfactory bulbs where it may cause neurotoxic insult. In the present study, 5 month old male C57 wild type (WT) and ApoE-/- mice were exposed to 100 $\mu g/m^3$ of spark generated Ni NPs (GFG-100 Palas, Germany) or filtered air in a whole-body exposure system for 5h/d, 5d/w for 6 weeks. Particle size was measured by a differential mobility analyzer coupled to a condensation nucleus counter and the exposure concentrations were determined gravimetrically. Blood was collected by facial vein bleeding and serum aliquots were made. Serum titers for antibodies, IgM and IgG, against neural proteins [neurofilaments (NF), glial fibrillary acidic protein (GFAP), myelin basic protein (MBP)], mitochondrial cardiolipin (CL) and oxidized-LDL (oxLDL) were detected using ELISA. Levels of IgM and IgG against neural proteins in WT controls (n=6) were only marginally above the tris-buffer blank. The majority of antibodies detected in Ni-NP exposed WT (n=7) and ApoE-/- mice were predominantly the IgG isotype. Titers of IgG in ApoE-/- controls (n=4) were comparable to Ni-NP-exposed WT and were significantly ($p<0.05$) different than WT-controls. Titers of IgM and IgG were significantly ($p<0.05$) different than all other groups for ApoE-/- exposed to NI-NP (n=4). Similar patterns were measured for IgG titers against CL and oxLDL. The detection of neuroantibodies suggests that Ni-NP exposure induces neurotoxicity. This may involve OS and mitochondrial damage as indicated by detection of IgG against CL and oxLDL. Ni is known to induce OS, lipid peroxidation and interfere with mitochondrial function, mechanisms involved in neurodegeneration. The greater sensitivity of ApoE-/- mice to these effects is consistent with their vulnerability to OS, autoimmunity, cardiovascular and neurodegenerative changes. (Supported by NIEHS R01 ES015495-01 at NYU).

1879 VANADIUM INDUCES NEUROTOXICITY IN DOPAMINERGIC NEURONAL CELLS *VIA* PROTEOLYTIC ACTIVATION OF PROTEIN KINASE C-DELTA: RELEVANCE TO ETIOPATHOGENESIS OF PARKINSON'S DISEASE.

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Environmental exposure to neurotoxic metals and pesticides is considered a risk factor in the etiology of Parkinson's disease (PD). In this regard, there is a growing concern that chronic exposure to welding fumes may have an association with increased incidences of PD. Welding fumes contain many different metals, and vanadium is typically present as vanadium pentoxide (V_2O_5), but possible neurotoxic effects of this metal oxide are not well characterized. Therefore, we examined the effect of V_2O_5 on a dopaminergic cell model of PD (N27 cells) and characterized the oxidative stress-dependent cellular events. Exposure to 3-300μM V_2O_5 for up to 12 hr resulted in a time- and dose-dependent increase in cytotoxic cell death in two independent assays: MTT and Sytox Green. The EC_{50} value of V_2O_5 was determined to be 37μM from the cytotoxicity data. Exposure to 40μM V_2O_5 resulted in a significant increase in reactive oxygen species (ROS) generation, as determined by the Apollo 4000 Free Radical Analyzer. The ROS generation was followed by ac-

tivation of mitochondrial-dependent proapoptotic events such as sequential activation of initiator caspase, caspase-9, and executioner caspase, caspase-3, in a time-dependent manner. Interestingly, we also found that V_2O_5 exposure induces proteolytic cleavage of native protein kinase Cδ (PKCδ, 72-74 kDa) to yield 41-kDa catalytically active and 38-kDa regulatory fragments. Pretreatment with caspase inhibitors Z-DEVD-FMK or Z-VAD-FMK blocked V2O5-induced proteolytic cleavage of PKCδ, demonstrating the role of caspase-3 in kinase proteolysis. Attenuation of caspase-3 and PKCδ activity by pharmacological inhibition prior to V_2O_5 treatment significantly suppressed increases in DNA fragmentation. Together, these results demonstrate V2O5 exerts a neurotoxic effect in a dopaminergic cell model of PD via caspase-3 dependent proteolytic activation of PKCδ, and suggest that vanadium neurotoxicity may play a role in the etiology of PD (supported by NIH grant ES10586)

1880 MODERATE PERINATAL ARSENIC HAS LONG-TERM AFFECTS ON LEARNING AND MEMORY BEHAVIOR AND ERK PHOSPHORYLATION.

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Arsenic (As) is one of the most common naturally occurring contaminants found in the environment. While most studies on As have focused on its carcinogenic potential, a few studies suggest that As can affect human cognitive development in both rodents and humans. The mechanism of As damage is yet to be elucidated; however, As has been shown to perturb the hypothalamic-pituitary-adrenal axis. Perturbation of this axis has been implicated in both cognitive damage and the promotion of carcinogenesis. One parameter of this axis, the glucocorticoid receptor (GR), has been shown to be altered by As. These receptors are found throughout the central nervous system but are concentrated in the hippocampus, an area of the brain of importance for learning and memory. Phosphorylation of Extracellular Signal Regulated Kinase (ERK) has also been implicated in learning and memory. Binding of corticoids to the GR can activate transcription of members of the RAS signaling pathway ultimately leading to phosphorylation of ERK. We examined the impact of perinatal exposure to 50 parts per billion As on learning and memory behavioral tests and ERK in the C57BL6/J mouse. Novel object task was used as a measure of visual and spatial learning and memory. One parameter scored was latency to reach the novel object in which As-exposed animals showed longer latency to reach novel object than controls. In the 8-Way Radial Arm Maze, a test for both working and reference spatial learning and memory, As animals had a significant increase in the number of errors compared to controls. Results from the behavioral studies suggest that moderate perinatal As can have long-lasting adverse effects on learning and memory behavior. In addition to the behavioral studies, measurements of phosphorylated and total ERK were taken. Preliminary results show that As animals have lower ratios of phospho/total ERK than controls. Taken together this data suggests that moderate levels of perinatal As can have a lasting impact on offspring.

1881 INORGANIC ARSENIC METHYLATION AND ACCUMULATION IN MOUSE BRAIN REGIONS.

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Dose-repeated oral exposure of mice to sodium arsenite have shown a dose-related distribution of As and of its methylated metabolites in the brain. While inorganic arsenic increased linearly with dose in organs such as liver, an opposite pattern was observed in the central nervous system (CNS) where dimethylarsenic (DMA) was the most abundant specie, suggesting that biomethylation was taking place. We investigated the expression of As3MT and the formation of monomethylarsenic (MMA) and DMA in the following regions of the mouse brain: cerebral cortex, hippocampus, striatum, mesencephalon, thalamus, cerebellum, hypothalamus, pons, medulla oblongata and hypophysis. CD-1 mice were given 0, 2.5, 5 and 10 mg/kg/day of sodium arsenite orally during 9 days. Animals were killed by cervical dislocation and brain dissected immediately and frozen in liquid nitrogen. The expression of *As3MT* was determined using RT-PCR and the accumulation of arsenic species was measured by hydride generation–atomic absorption spectroscopy after column chromatographic separation of inorganic arsenic and its metabolites. We observed the expression of *As3MT* mRNA in all brain regions. Methylated arsenic species, especially DMA was the most abundant form in all regions with the exception of the hypophysis where no methylated forms were found, in the animals that received the highest dose (10 mg/kg/day). Arsenic biomethylation in the CNS might alter the homeostasis of key molecules in brain biochemistry such as S-adenosyl methionine and its intermediate metabolites, including methionine, choline, folate, and vitamins B6 and B12, some of which are important substrates or cofactors in neurotransmitter metabolism.

1882 GENE EXPRESSION BIOMARKERS ASSOCIATED WITH NEUROTOXICITY IN MICE EXPOSED WITH SILVER NANOPARTICLES BY INHALATION.

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Nanomaterials are in widespread use in the field of science, technology and medicine. Among them silver nanoparticles (AgNP) are emerging as one of the fastest growing product categories in the nanotechnology industry. According to the data available to date, it is believed that the use of nanomaterials in the consumer and industrial products may cause some health problems in humans. The transport of nanoparticles directly to the brain through the olfactory bulb poses serious concern for the neurotoxicity. Currently, however, little is known about the adverse effects of silver nanoparticles to human health. Our aim was to profile global gene expression in the cerebrum of the mice exposed to AgNP by inhalation and to develop biomarkers for the evaluation of AgNP exposure in mice. Groups of mice were exposed to AgNP (CMD 22 nm) at a concentration of 1.1 mg/m3 for 2 weeks (6 hrs/day; 5 days/week) in a nose-only exposure system. An additional group of mice were treated with AgNP as described and allowed to recover for 2 weeks in a fresh air. Tissue distribution of AgNP was monitored using ICP/Mass spectrometry. Total RNA was extracted from the cerebrum and cerebellum and microarray hybridization was performed using Affymetrix GeneChip. A total of 47 genes from the cerebrum and 22 genes from the cerebellum was identified and designated as AgNP-responsive. The expression of some genes were monitored in the whole blood obtained from AgNP-exposed mice and discussed the possibility to be used as biomarkers for AgNP exposure as well as their effects on the brain.

1883 CHARACTERIZATION OF THE EFFECT OF METHYLMERCURY ON *CAENORHABDITIS ELEGANS.*

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Mercury (Hg) is a toxicant that displays neurotoxic effects via unknown molecular mechanisms, particularly when exposure occurs during early development. Humans are exposed to methylmercury (MeHg) upon its consumption in seafood, absorption, and passage through both the blood-brain and placental barriers. *Caenorhabditis elegans*' utility as a bioassay for toxicants has been recently exploited, but our studies are the first to expose *C. elegans* to MeHg. We hypothesize *C. elegans* is a valuable model for studying the effects of MeHg on the nervous system delineating molecular mechanisms of toxicity and genetic susceptibility. Upon treatment and subsequent observation of 200 worms under treatment conditions at least 20 times, we observed that MeHg is toxic to *C. elegans*. A decrease in lethality is observed when exposure occurs farther along in development (L4, LC50=2.3mM MeHg) compared to earlier exposure (L1, LC50=1.3mM MeHg). As L4 MeHg exposure duration increases, toxicity increases (30-minute exposure yields LC50=2.3mM MeHg, 6-hour exposure yields LC50=0.43mM MeHg, and 15-hour exposure yields LC50=0.34mM MeHg). Brood size is not affected by MeHg, but a delay in egg-laying coupled with decreased size immediately following exposure indicate a developmental delay, the delay at LC50 treatment conditions is one day. Spontaneous and poke-induced movement indicated no alterations in behavior. Fluorescent analysis of neuronal morphology upon MeHg exposure was not associated with changes in dopamine, acetylcholine, or GABA. Studies in additional neurotransmitter systems, quantitative behavioral tests, and genetic screens are forthcoming. Our research indicates that *C. elegans* is a useful tool for studying MeHg toxicity and will allow us to examine molecular mechanisms and genetic susceptibility for the effects of this toxin.
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1884 INVESTIGATION OF MANGANESE TRANSPORT IN *C. ELEGANS.*

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Manganese (Mn) is an essential trace element for normal human development and vital enzymatic activities. However, occupational exposure to high levels of Mn has been implicated in a Parkinsonian-like syndrome known as manganism, affecting

the dopaminergic system. Since the initial discovery of Mn-induced pathogenesis, much effort has been put forth in order to study the uptake and potential adverse effect of manganism. In the present study, we use *C. elegans* to understand the mechanism of Mn transport across biological membranes. It was shown in mammalian systems that Mn^{2+} can cross the blood-brain barrier (BBB) through the divalent metal transporter DMT1/NRAMP2. The *C. elegans* genome encodes three putative DMT1 orthologues (SMF-1, SMF-2, SMF-3), which are differentially expressed. We report here the sensitivity to Mn exposure exhibited by the three knockout strains *smf-1(eh5), smf-2(gk1330), smf-3(ok1035)* by examining the lethality of these knockouts to various doses of Mn (0 – 2 M $MnCl_2$). Initial results indicate that Mn is transported and can exert a different effect on the knockout compared to wild type (wild type: EC_{50} = 48.00 ± 1.19 mM vs. *smf-2* KO: EC_{50} = 29.42 ± 1.15 mM ; $p < 0.05$). Since the differences in response to Mn exposure may be due to the specificity of uptake amongst the three orthologues, the expression pattern of the three *smf* genes, and the localization of the SMF proteins tagged with GFP is presently investigated. Studies to date suggest that Mn-induced toxicity is governed by Mn transport in *C. elegans* and is a function of differential localization and functionality of the three DMT1 isoforms. Given the similarity of DMT1 gene between humans and *C. elegans*, these results suggest that *C. elegans* is a valuable model to study Mn transport and toxicity in humans.
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1885 INVESTIGATION OF MANGANESE TOXICITY IN PD GENES DEFICIENT C. ELEGANS.

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Manganese (Mn) is an essential trace element for normal human development and vital enzymatic activities. However, occupational exposure to high levels of Mn has been implicated in a Parkinsonian-like syndrome known as manganism, affecting the dopaminergic system. We hypothesized that the commonalities in clinical and physiological symptoms exhibited in both Parkinson's Disease (PD) and manganism patients, at least partially, rely on common molecular pathways. In the present study, we used C. elegans strains mutated for PD and dopaminergic neuron-specific genes and evaluated their sensitivity to acute Mn exposure. We first observed that the C. elegans dopaminergic system is sensitive to Mn exposure in a dose-dependent manner. Confocal microscopy shows that a 30' treatment of 0.5, 5, 50, or 500 mM MnCl2 specifically leads to dopaminergic neuron degeneration, while sparing other neurotransmitter systems, such as GABAergic or cholinergic neurons. We further established that the dopamine transporter knock-out dat-1(ok157) is 5 times more sensitive to Mn exposure than wild-type worms (wild type: EC50 = 45.72 +/- 1.19 mM vs. dat-1: EC50 = 9.16 +/- 1.11 mM, $P < 0.0005$). In addition, we cloned the C. elegans PD genes orthologues pink-1, djr-1.1 and djr-1.2 in frame with GFP and report here their expression pattern. We will also present preliminary data on the sensitivity to Mn acute exposure of mutants lacking these genes. Combine, these results establish C. elegans as a suitable model for studies on genetic pathways involved in manganese toxicity. Additionally, they support the hypothesis of congruence between PD and manganism at the molecular level.
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1886 METHYLMERCURY INDUCED OXIDATIVE INJURY AND INFLAMMATORY RESPONSE IN RAT PRIMARY ASTROCYTES: ATTENUATION BY ANTIOXIDANTS.

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Methylmercury (MeHg) is a major neurotoxicant that continues to pose a risk to human health. Astrocytes accumulate MeHg and play a prominent role in mediating MeHg toxicity. MeHg has been shown to interfere with multipe intracellular targets, thereby contributing to pathogenic processes typical of neurodegenerative disorders. We tested the hypothesis that MeHg treatment in neonatal rat primary astrocyte cultures results in increased oxidative injury and inflammatory response, which, in turn is suppressed by antioxidants action. Treatment of astrocytes for 1 or 6 hours with MeHg (5 or 10 uM) caused a significant ($p<0.05$) increase in F2-isoprostanes (F2-IsoPs), lipid peroxidation biomarkers of oxidative damage. Consistent with this observation, MeHg induced a concentration-dependant reduction in the inner mitochondrial membrane potential ($\Delta\Psi m$), assessed with tetramethylrhodamine ethyl ester (TMRE). Astrocytes exposed to 5 μM MeHg also showed time-dependent increase in COX-2 protein expression (8 fold increase at 6 hours). Additionally, the stimulatory effect of MeHg on COX-2 expression was accompanied by significant ($p<0.05$) elevation of the proinflammatory mediator prostaglandine E2 (PGE2) (both at 2 and 6 hours). When astrocytes were pretreated for 30 min before MeHg exposure with the antioxidants alpha-phenyl-N-tert-butylnitrone (PBN, 100 uM) or Trolox (100 uM), a significant attenuation in MeHg-induced increases in F2-IsoPs and dissipation of $\Delta\Psi m$ was noted. Furthermore, PBN or Trolox pretreatment suppressed the MeHg-induced expression of COX-2 and the PGE2 at 6 hours following the exposure. Thus, MeHg-induced synergistic mechanisms of oxidative injury and inflammation leading to cellular dysfunction and cell death are readily suppressed by antioxidant effect of PBN or Trolox. Taken together, the studies establish that oxidative stress and inflammatory reactions play a major role in MeHg-induced neurotoxicity (Supported by NIEHS ES07331).

1887 EBSELEN ATTENUATES MANGANESE-INDUCED OXIDATIVE INJURY IN RAT PRIMARY ASTROCYTES.

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Chronic exposure to excessive manganese (Mn) results in a movement disorder termed manganism, which resembles Parkinson's disease (PD). The pathogenic mechanisms underlying this disorder are not revealed. Studies implicate astrocytes as an early target and mitochondria as an intracellular target for Mn neurotoxicity, where it disrupts oxidative phosphorylation and increases the generation of reactive oxygen species. In the present study, we investigated the effects of Mn on astrocytic mitochondrial function and effects of antioxidant ebselen against Mn. Primary astrocytic cultures were prepared from cerebral cortices of one-day-old Sprague-Dawley rats. Cultures were grown to confluency in MEM with 10% horse serum. Cells were treated with Mn at 100, 500 and 1000μM with or without ebselen at 10μM. Western immunoblotting analysis was employed. The potentiometric dye TMRE was used to assess the role of Mn on mitochondrial inner membrane potential ($\Delta\Psi m$). The results indicate that expression of cyclooxygenase-1 (COX-1) altered significantly ($P<0.05$) from 30 min to 6 h exposure to Mn, COX-2 expression increased ($p<0.05$) until 6 h exposure to Mn. Ebselen totally inhibited COX-1 expression and partially reversed COX-2 expression ($p<0.05$). Ebselen pretreatment attenuated the Mn-induced reduction of DJ-1 and activation of AIF (apoptosis-inducing factor) and caspase-3 ($p<0.05$). Mn dissipated the $\Delta\Psi m$ as evidenced by decrease in mitochondrial TMRE fluorescence (1 h) ($p<0.01$); $\Delta\Psi m$ dissipation was reversed significantly with ebselen ($p<0.05$). These results are consistent with the hypothesis that Mn exerts its neurotoxicity via oxidative stress and that its toxicity is associated with the dissipation of $\Delta\Psi m$ and activation of apoptotic cascades. These results suggest that (1) activation of COX, caspase-3 and AIF is involved in Mn-induced neurotoxicity via mitochondrial-dependent pathways, (2) expression changes of DJ-1 upon Mn exposure may be involved mediating the neurotoxicity of this metal, and (3) ebselen can attenuate Mn neurotoxicity in cultured astrocytes.

1888 ANTIOXIDANTS REDUCE MANGANESE-INDUCED MITOCHONDRIAL IMPAIRMENT IN CULTURED ASTROCYTES.

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Manganese (Mn) is essential for maintaining the proper function and regulation of multiple biological processes. In the brain, Mn is an important cofactor for a variety of enzymes, including the anti-oxidant enzyme superoxide dismutase, as well as enzymes involved in neurotransmitter synthesis and metabolism. However, Exposure to excessive amounts of Mn is associated with a variety of psychiatric and motor disturbances, which resembles Parkinson's disease. The pathogenic mechanisms underlying this disorder are not fully understood. Several lines of evidence implicate astrocytes as an early target of Mn neurotoxicity. In current study, we investigated the hypothesis that cytotoxicity of Mn in astrocytes is via mitochondrial control of apoptosis. Primary astrocyte cultures were prepared from cerebral cortices of one day old Sprague–Dawley rats. Cells were pretreated for 30 min with Ebselen 10 μM or Trolox 100μM,then treated with Mn at 0, 500 μM for 30min and 1, 2, or 6h. Mn toxicity in astrocytes was assessed by lactate dehydrogenase (LDH) leakage into the culture medium. Apoptosis was assessed by measuring caspase-3 and –7 activities. The potentiometric dye tetrmethylrhodamine ethyl ester (TMRE) was used to assess the effect of Mn on astrocytic mitochondrial membrane potential ($\Delta\Psi m$). Our results show that Mn results in significant LDH releasing and activation of caspase-3 and -7 ($p<0.05$ by ANOVA), the effects of Mn can be significantly decreased by Ebselen or Trolox ($p<0.05$). Mn treatment (1 and 6 hrs) significantly ($p<0.01$) dissipated the $\Delta\Psi m$ in astrocytes as evidenced by a decrease in mitochondrial TMRE fluorescence, which also was reversed by Ebselen or Trolox. These results indicate that Mn exerts its neurotoxicity through oxidative reaction via mitochondria-dependent pathway and antioxidants might be potential therapeutic reagents against Mn-induced toxicity.

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1889 MECHANISMS OF ESTROGEN-MEDIATED NEUROPROTECTION IN MANGANESE-INDUCED NEUROTOXICITY.

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While manganese (Mn) is an essential element and is required for many biological functions and enzyme activities, excessive chronic exposure to Mn leads to neurological disorders such as Parkinsonism and Manganism. Although the mechanism of Mn-induced neurotoxicity has not been completely established, recent evidence suggests that glial cells play a crucial role in mediating its neurotoxicity. Estrogens (E2) have a broad spectrum of neural functions in the CNS including synaptic plasticity and developmental processes. Several lines of evidence suggest that E2 is neuroprotective in animal and cell models. Herein, we studied the ability of E2 to afford neuroprotection in a Mn-induced neurotoxicity model. Primary neuronal and glial cell cultures from rat cortical regions were isolated from rat embryonic and postnatal cortical regions and cultured for 1 to 3 weeks, respectively. E2 receptors, α and β were expressed in both astrocytes and neurons. E2 (10 nM) significantly ($p<0.05$) attenuated astrocytic cytotoxicity (alamar blue assay) associated with 12 hr treatment with 1 mM Mn. To better understand the cellular mechanisms of downstream activation of E2 receptor in Mn neuroprotection, we studied the role of MAPK, as Mn is known to stimulate this pathway. Mn activated ERK phosphorylation within 10 min in rat astrocytes (37% increase) and E2 inhibited Mn-induced ERK phosphorylation, whereas p-38 phosphorylation was unaltered. On the other hand, neither Mn nor E2 did activate ERK phosphorylation in neuronal cultures. These results suggest that the protective effect of E2 on Mn-neurotoxicity is mediated, at least in part, by alteration in MAPK signal pathway in astrocytes.
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1890 CYTOPROTECTIVE MECHANISMS OF DICARBONYL TRAPPING ANTI-DIABETIC DRUGS.

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Carbonylation is an irreversible, non-enzymatic modification of proteins. Diabetes is a disease among several others that have been linked with the accumulation of carbonylated proteins in tissues. In Diabetes, dicarbonyls (glyoxal and methylglyoxal) are generated by the autoxidation of reducing sugars (glucose) which react with protein and eventually lead to the formation of advanced glycation end-products (AGEs). Anti-diabetic drugs containing thiol or amine functional groups have been suggested to act therapeutically by preventing protein carbonylation by trapping glyoxal (GO) to form non-toxic adducts. This study seeks to determine the mechanism by which anti-diabetic drugs prevent GO toxicity in a cell-free system as well as in isolated rat hepatocytes. In a cell free system, the GO trapping ability of the drugs was measured by following GO disappearance using Girard's Reagent T. For the thiol-containing drugs, the order of effectiveness for GO trapping was penicillamine > cysteine > N-acetyl cysteine, whereas for the amine-containing drugs, the order of effectiveness for GO trapping was aminoguanidine >> pyridoxamine > metformin. Furthermore, aminoguanidine, N-acetyl cysteine and penicillamine at concentrations equimolar to GO prevented GO-induced hepatocyte cytotoxicity and removed GO as measured spectrophotometrically. However, other scavenging drugs such as pyridoxamine, hydralazine or metformin did not prevent GO-induced cytotoxicity by trapping GO, but instead prevented cytotoxicity by acting as a mitochondrial vitamin, lipid antioxidant or reactive oxygen species scavenger, respectively.

1891 PRECLINICAL INVESTIGATION OF FIBRODYSPLASIA AND ITS RELEVANCE TO HUMAN MUSCULO-SKELETAL SYNDROME.

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The clinical development of matrix metalloproteinase (MMP) inhibitors for the treatment of diseases such as cancer and arthritis, including the broad spectrum inhibitors Marimastat and Prinomastat, has been curtailed by musculo-skeletal syndrome (MSS), a painful condition of the joints. The mechanism underlying this adverse lesion is not well understood. To further our knowledge we assessed the response of rats and dogs to a broad spectrum MMP inhibitor. We found that rats and dogs developed lesions that were characterised by a fibrodysplasia (FD) as evident by fibroblast proliferation, increased collagen synthesis and inflammatory cell infiltration. The pattern of onset and characteristics of fibrodysplasia were consistent with the MSS seen clinically. In the rat, the tarsal joint was the most susceptible tissue, although the FD was also evident in synovial tissues, tendons and ligaments with onset of appearance between 14 and 28 days. In the dog the most susceptible tissue was the subcutaneous connective tissues, with skin thickening and stiffening, particularly in the dorsal cervical region, which also occurred between 14 and 28 days of dosing. FD was also observed in the connective tissue of other organs in the dog, including the heart and eye, and there were disturbances in wound healing. The dog was more sensitive than the rat, as determined by systemic exposure comparison. These data show that both rat and dog are susceptible to MMP inhibitor-induced FD, however, there are species differences in sensitivity and profile of organ involvement. It is important to understand the relevance to MSS in man of these findings, in terms of mechanistic association and organ involvement, and to identify compounds with suitable profiles that do not cause this lesion.

1892 DIAGNOSTIC CRITERIA FOR SELECTED PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR (PPAR) AGONIST-INDUCED MESENCHYMAL LESIONS IN THE RAT.

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In 2005, the Health and Environmental Sciences Institute (HESI) PPAR Agonist Project Committee was established to advance research on and to understand the modes of action and human relevance of this emerging rodent tumor data for PPAR agonists. A Pathology Working Group (PWG) was convened to develop consensus on morphologic criteria for tumor diagnoses and consistency of diagnoses across multiple studies for liposarcomas/fibrosarcomas in rats.
Participating institutions confidentially submitted multiple unstained slides from 99 rats for hematoxylin and eosin (H&E) staining and pathological review. Selected lesions were used for special histochemical and immunohistochemical staining. Lesions were most frequently submitted from subcutaneous and adipose tissues. Diagnoses involving proliferative lesions included fibrosarcoma, liposarcoma, malignant schwannoma, lipoma, hibernoma, fibroma, sarcoma, NOS, and hyperplasia, adipose tissue. Induced lesions often had myxomatous changes within the stroma that stained positively with Alcian blue. Distinction between PPAR-induced fibrosarcomas and liposarcomas was obscured in some cases by pleomorphic cell morphology, vacuolar changes of fibroblasts, and a prominent fibrosarcomatous element within liposarcomas.
For diagnosis of PPAR-induced lesions, final recommendations by the PWG consisted of slight modifications of the nomenclature and diagnostic criteria previously established by the STP. The routine use of histochemical and/or immunohistochemical stains was not recommended for the classification of these poorly differentiated sarcomatous neoplasms.

1893 DIAGNOSTIC CRITERIA FOR SELECTED PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR (PPAR) AGONIST-INDUCED VASCULAR LESIONS IN THE MOUSE.

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In 2005, the Health and Environmental Sciences Institute (HESI) PPAR Agonist Project Committee was established to advance research on and to understand the modes of action and human relevance of this emerging rodent tumor data for PPAR agonists. A Pathology Working Group (PWG) was convened to develop a consensus on morphologic criteria for tumor diagnoses and consistency of diagnoses across multiple studies for vascular changes and vascular proliferative lesions in mice.
Participating institutions confidentially submitted unstained slides from 420 mice for hematoxylin and eosin (H&E) staining and pathological review. Tissues were most commonly submitted from the skin/subcutaneous tissue, liver, spleen, heart and lymph node. Diagnoses involving proliferative lesions included hemangiosarcoma, hemangioma, angiolipoma, and angiomatous hyperplasia. Non-proliferative lesions included atrial thrombosis in the heart, congestion with hemorrhage in lymph nodes, and angiectasis, thrombosis, hemorrhage, and/or congestion in various organs
Lesions fairly unique to treatment with PPAR agonists included angiomatous hyperplasia and angiolipoma. In a few tumors that would have otherwise been classified as angiolipoma, regions of the vascular component appeared to be malignant,

and were classified as hemangiosarcomas. With the exception of these two lesions, it was concluded by the PWG that the diagnostic criteria established by the STP for vascular neoplasms could be applied to PPAR-induced lesions, with slight modifications in nomenclature and criteria.

1894 STABILITY OF THE MYCOPHENOLIC ACID ACYL GLUCURONIDE IN BIOLOGICAL MATRICES.

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Acyl glucuronides (AG) are controversial in toxicology due to their ability to form protein adducts with liver and plasma proteins, but their role in adverse drug reactions (ADRs) remains unclear. AGs have the ability to form protein adducts due to their property of intermolecular acyl migration exposing a reactive aldehyde that forms covalent bonds with cellular macromolecules. While glucuronidation is generally considered a detoxification mechanism, numerous NSAIDS forming AGs have been removed from the market due to ADRs. Mycophenolic acid (MPA) is an immunosuppressant used in renal transplant patients that exhibits delayed onset diarrhea. These experiments examine the reactivity of the AG of MPA (acMPAG) by evaluating its stability in phosphate buffer and biological matrices.
AcMPAG (Novartis) was added to either phosphate buffer (pH 4, 7.4, 9) or human plasma and incubated at 37°C. At specified time points, aliquots were either quenched by acified acetonitrile or subject to hydrolysis by β-glucuronidase. After quenching with acidified acetonitrile, samples were centrifuged and the supernatant was dried under N2 gas then reconstituted in 30%ACN/70% mobile phase. HPLC analysis employed on a RP C18 column with UV detection set at 250nm. The matrix dependant degradation of acMPAG was determined to access acyl migration and hydrolysis.
The purity of acMPAG found that the initial solution contained 80% acMPAG and 20% isomeric conjugates. The acMPAG in pH 4 buffer had a t1/2 of 36 h compared to pH 9 (4.5 h) and pH 7.4 (15.5 h). Compared to the phosphate buffers, the t1/2 in human plasma (5 h) was significantly less than pH 7.4 buffer. The decreased t1/2 of the acMPAG within human plasma indicates that the plasma proteins appear to destabilize the AG. The relative reactivity of acMPAG suggests it is a moderately reactive AG but may also lead to covalent binding to tissues in vivo.

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1895 PHARMACOKINETICS AND TOXICITY TO RATS OF SR13668, A NOVEL CANCER CHEMOPREVENTIVE AGENT.

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SR13668 (2,10-Dicarbethoxy-6-methoxy-5,7-dihydro-indolo[2,3-b]carbazole), a product of rational drug design based on indole-3-carbinol analogs, is active in vivo against breast, prostate, and ovarian tumor xenografts in mice. Its anticancer mechanism is consistent with selective inhibition of the phospho-Akt signaling pathway. Definitive pharmacokinetics and subchronic toxicity studies were performed in male and female Sprague Dawley rats, with SR13668 administered by oral gavage in PEG400:Labrasol (1:1). Dose levels were 1, 10, and 30 mg/kg for pharmacokinetics, and 0, 30, 150 and 1000 mg/kg/day in the 28-day toxicity study. At lower doses, up to 150 mg/kg, AUC and Cmax increase with dose level, and are higher in female than male rats. The oral bioavailability is ~15-25%. Apparent volume of distribution and clearance are higher in male rats (~8 l/kg and 800 ml/hr/kg) than in females (~6 l/kg and 660 mg/hr/kg). The elimination half life is 6-9 hr. SR13668 is essentially 100% plasma protein bound, and the ratio of drug concentration in blood/plasma is ~3. SR13668 also concentrates (> 20-fold) in lung tissues relative to blood. Subchronic rat studies suggest that SR13668 is well tolerated, with minimal effects observed in all dose groups. The maximum tolerated dose level was determined to be >1000 mg/kg for 28 days. Unlike other inhibitors of phospho-Akt, SR13668 did not increase blood glucose levels after single or multiple dose administration, suggesting a better safety profile. Further preclinical development of SR13668 is underway in dogs, and an exploratory IND is being planned to evaluate human pharmacokinetics in a clinical study (Supported by RAPID Program and NCI Contract HHSN261200433005C (N01-CN-43305)).

1896 USING KINASE SELECTIVITY PROFILES TO PREDICT *IN VITRO* AND *IN VIVO* BONE MARROW TOXICITY.

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The fundamental roles that kinases play in various cellular functions represents a tremendous opportunity for pharmacologic intervention, but also often produce unwanted and unexpected toxicities when inhibited. For example, inhibition of certain kinases that are involved with hematopoietic progenitor stem cell proliferation and differentiation can cause cellular toxicity leading to bone marrow ablation. Because most small molecule kinase inhibitors (SMKIs) target the highly conserved ATP binding pocket, many SMKIs are not selective and are prone to non-pharmacologically related toxicities. Internal experience from multiple kinase programs has demonstrated that a large proportion of Roche SMKIs cause bone marrow ablation in vivo, yet our understanding of which kinases are responsible for this toxicity and our ability to predict this result with an in vitro assay is limited. To address this need, a training set of 50 SMKIs were analyzed for trends across the kinase inhibitory profiles versus in vivo bone marrow toxicity. Inhibition of a number of kinases was identified to be correlated with in vivo bone marrow ablation, including AAK1, Aurora A, JAK1 & 2 (p-values of 0.004, 0.0001, 0.004, and 0.003, respectively). In addition, a rodent CFC-GEMM progenitor in vitro toxicity assay based upon cellular proliferation and the release of intracellular ATP has been evaluated for its ability to predict in vivo hematopoieitic toxicity. A validation study consisting of thirty proprietary and non-proprietary compounds demonstrated that the CFC-GEMM assay had a high correlation with in vivo bone marrow ablation (23/30, 76%). Current work is focused on increasing the number of SMKIs screened with an expanded kinase inhibitory platform as well as with the CFC-GEMM progenitor cell assay to obtain a better mechanistic understanding of the individual kinases critical to hematopoesis, and determine if kinase inhibitory profiles can predict in vitro and in vivo hematopoietic toxicities.

1897 TOLERABILITY OF AN INHALED ANTISENSE OLIGONUCLEOTIDE IN MOUSE AND MONKEY.

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The tolerability of nebulized ISIS 369645, a 2'-O-methoxyethyl phosphorothioate (2'-MOE) antisense oligonucleotide to human IL-4Rα, was evaluated in monkey and mouse. Inhaled doses of 0.5, 1.5, 5 and 15 mg/kg/wk were administered to monkeys for 4 weeks via facemask. In mice, inhaled doses of 0.4, 2, 10 and 50 mg/kg/wk were administered for 13 weeks via nose-only inhalation. For both studies, loading doses were administered on Days 1, 3, 5 and 7 with a subsequent once-weekly dose regimen for up to 3 months and included a 13-week recovery period. Inhalation exposure resulted in the largest portion of the dose being deposited locally in lung and the tracheobronchial lymph nodes. Toxicokinetic data confirmed continuous and dose-dependent exposure to ISIS 369645. Systemic distribution was estimated to be 2–10% of inhaled dose and only very low concentrations were measured in the known primary target organs for oligonucleotide distribution, kidney and liver. Thus, exposure to systemic tissues was well below levels associated with target organ effects for parenterally administered 2'-MOE oligonucleotides. There were no treatment-related signs of clinical toxicity, mortality or body weight change. The toxicity profiles of mice and monkeys treated with inhaled ISIS 369645 were similar, producing no significant local irritation at any dose level tested. The most prominent change associated with inhalation exposure was a dose-dependent increase in size and number of macrophages in the alveoli and tracheobronchial lymph nodes. This finding was minimal to mild in severity, local to the site of delivery and reversible. These changes are consistent with the role of macrophages in the uptake and clearance of oligonucleotide. There was no associated change in pulmonary function as evaluated at multiple time points after repeated administration in monkeys. Because of the absence of meaningful local reactions at the site of exposure, repeated administration by inhalation was determined to be a well-tolerated route of administration.

1898 PRECLINICAL SAFETY EVALUATION OF INHALED S-NITROSOGLUTATHIONE.

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S-nitrosoglutathione (GSNO) is an endogenous nitric oxide donor and serves key homeostatic functions in the lung including dilation of human airways and maintaining ciliary function. Decreased pulmonary levels of GSNO have been reported in obstructive lung diseases including asthma. Experimental studies of GSNO have shown bronchodilatory activity and replacement therapy may be beneficial to asthma and cystic fibrosis patients.
In support of clinical development for GSNO, safety studies were conducted using conventional inhalation toxicology methods including acute and multi-dose protocols in rats & dogs. Safety pharmacology studies were also performed.
In acute studies, the No Observable Adverse Effect Level (NOAEL) for rats and dogs were 320 mg/kg and 30 mg/kg, respectively. In both cases this dose represented the highest aerosol concentration that could be administered using available inhalation technology.

In a multi-dose rat study with GSNO minimal effects were seen. The two most notable observations were evidence of increased red cell turnover (increased reticulocytes) and squamous metaplasia in the larynx. Laryngeal metaplasia is reflective of a minimal irritant response and is often considered an adaptive change in the rat respiratory tract. Dogs did not manifest a similar metaplasia suggesting that such irritancy may be related to a uniquely sensitive laryngeal epithelium in rat.
In the dog there was no evidence of notable toxicity at the maximum dose. In safety pharmacology studies, no effects on heart rhythm or QTc were detected; however, increases in heart rate were observed and considered to reflect the pharmacology of GSNO.
These data demonstrate that supraphysiologic levels of inhaled GSNO are well tolerated in multiple species and replacement therapy could provide a safe therapeutic strategy in the treatment of obstructive disease.

1899 OCCURRENCE AND BEHAVIOR OF ANTI-INFLAMMATORY DRUG NAPROXEN IN AQUATIC ENVIRONMENT.

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Recently, environmental water in pharmaceuticals and personal care products has become public concern. Naproxen, (S)-6-methoxy-alpha-methyl-2-naphthaleneacetic acid, is one of the non-steroidal anti-inflammatory drugs and is extremely used as non-prescription drugs. Naproxen has been detected at high frequency and under a few micro-gram per litter in urban river water and sewage treatment plants (STPs). However, a little is known about degradation and chiral inversion of naproxen in the aquatic environment. The objectives in this research were to clarify (1) degradation and the enantiomer ratio (ER, R-form/S-form) of naproxen in STPs, (2) degradation of naproxen under laboratory condition by activated sludge, and (3) changing of the ER of naproxen in river water.
Naproxen and a degradation products 6-O-desmethyl-naproxen, DM-naproxen, in water samples were extracted by solid-phase extraction under acidic conditions. The analytes were determined by GC-MS after trimethylsilyl derivatization. Enantioselective separation of naproxen was performed by LC-MS/MS on a chiral separation column.
Naproxen was detected in influents and effluents of STPs at concentrations of up to 1030 and 228 ng/L, respectively. The concentrations of DM-naproxen in influents and effluents were up to 458 and 558 ng/L, respectively. (R)-Naproxen was not detected in influents of STPs, whereas it was present in effluents of STPs at the ER from 0.10 to 0.14. Under laboratory conditions with active sludge of STPs, degradation of (S)-naproxen to DM-naproxen and chiral inversion of (S)-form to (R)-form were observed. In the river waters, which effluents of STPs flows into, naproxen and DM-naproxen were detected at concentrations of up to 138 and 160 ng/L, respectively, and the ER of naproxen were ranged from 0.02 to 0.17. These results suggest that the absence of (R)-naproxen in river waters reveals the inflow of untreated sewage.

1900 ROLE OF ENDOCYTIC ARK/PRK KINASES IN ADRIAMYCIN RESISTANCE.

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Adriamycin is an anticancer drug that is widely used in a clinical setting. In cancer chemotherapy with adriamycin, both the natural and the acquired resistance of cancer cells pose serious problems, as do the adverse effects of the drug. To elucidate the mechanism of acquired resistance to adriamycin, we searched for genes that, when overexpressed, render Saccharomyces cerevisiae resistant to adriamycin. We identified AKL1, a gene of which function is unknown, is considered, nonetheless, to be a member of the Ark/Prk kinase family, which is involved in the regulation of endocytosis, on the basis of its deduced amino acid sequence. Among tested members of the Ark/Prk kinase family (Ark1, Prk1, and Akl1), overexpressed Prk1 also conferred adriamycin resistance on yeast cells. Prk1 is known to dissociate the Sla1/Pan1/End3 complex, which is involved in endocytosis, by phosphorylating Sla1 and Pan1 in the complex. We demonstrated that Akl1 promotes phosphorylation of Pan1 in this complex and reduces the endocytic ability of cell, as does Prk1. Sla1- and End3-defective yeast cells were also resistant to adriamycin, and overexpression of Akl1 in these defective cells did not increase the degree of adriamycin resistance, suggesting that Akl1 might reduce adriamycin toxicity by reducing the endocytic ability of cells via a mechanism that involves the Sla1/Pan1/End3 complex and the phosphorylation of Pan1. In mammalian cells, adaptor-associated kinase 1 (AAK1) is a member of the Ark/Prk kinase family, and overexpression of AAK1 has been reported to reduce the endocytosis. We also found that HEK293 cells that overexpressed AAK1 were resistant to adriamycin. Our findings suggest that phosphoregulation of endocytosis by Ark/Prk kinase family might be closely involved in the mechanism of adriamycin resistance.

1901 DRUG INTERFERENCE WITH BIOTRANSFORMATION OF BISPHENOL A AND NONYLPHENOL IN RATS.

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4-Nonylphenol (NP) and Bisphenol A (BPA) are endocrine disruptors that have been detected in human samples. The major concern regarding exposure to these environmental pollutants is their estrogenic effects observed in both in vitro and in vivo studies. These chemicals are metabolized to inactive metabolites by conjugation to glucuronic acid. Many factors including co-exposure to other xenobiotics are susceptible of reducing these glucuronidation rates. This study aimed to identify and characterize the potential metabolic interaction between 14 widely used drugs and these two endocrine disruptors. The identification phase was done by incubating NP and BPA alone or in combination with drugs in suspensions of freshly isolated rat hepatocytes at concentrations 50 times higher than the reported Cmax in human. Metabolic rates were quantified by HPLC coupled to a fluorescence detector. Both pollutants exhibited similar inhibition profiles by drugs. Inhibition of NP and BPA biotransformation was seen with most of the drugs. Naproxen (18.7mM), salicylic acid (24.5mM), carbamazepine (1.9mM) and mefenamic acid (1.45mM) inhibited over 50% of NP and BPA metabolism. Characterization of the inhibition observed with naproxen and salicylic acid was attempted with rat liver microsomes. Naproxen was found to competitively inhibit the glucuronidation of NP (Ki=581.7μM) and BPA (Ki=848.3μM). Surprisingly, salicylic acid did not show inhibition of glucuronidation rates at 1000μM in microsomal incubations. This finding suggests that other mechanisms of action may be involved in the inhibition of NP and BPA. In conclusion, many drugs inhibit the glucuronidation of NP and BPA. Drug consumption should therefore be more carefully addressed in the health risk assessment of these contaminants as well as environmental pollutants in general.

1902 SPECIES-DEPENDENT PHARMACOKINETICS OF CMX001, AN ORALLY AVAILABLE PRODRUG OF CIDOFOVIR FOR TREATMENT OF SMALLPOX.

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CMX001 is an orally available lipid conjugate prodrug of cidofovir, a nucleotide analog approved for treatment of CMV but active against many double-stranded DNA viruses. The clinical utility of cidofovir is limited by poor bioavailability, requiring intravenous infusion and complicated by nephrotoxicity. CMX001 is under development for treatment of smallpox, a potential bioterrorist threat. CMX001 provided complete protection from mortality in mouse and rabbit models of poxvirus infection but was less efficacious in a cynomolgus monkey model. The pharmacokinetics of CMX001, cidofovir, and CMX064, the major (inactive) metabolite, were evaluated in single and multiple oral dose studies in mice, rats, rabbits and cynomolgus monkeys. Following oral administration, CMX001 was well absorbed in mice, rats and rabbits with Tmax occurring in 1 to 6 hours. In these species, Cmax and AUC increased about 1-3 fold greater than in proportion to dose. Slight accumulation was observed between the first dose and steady state. By contrast, values for Cmax and AUC in monkeys were about 5 to 10-fold lower and Tmax occurred earlier, at 0.5 to 2 hours, both likely a reflection of the rapid metabolism of CMX001. The terminal phase half-life ranged from 3 to 9 hours in rodents and rabbits but was only 1 hour in monkeys. Values for cidofovir Tmax ranged from 8 to 12 hours in mice, rats and rabbits but only 4 hours in monkeys. Cmax and AUC increased approximately in proportion to dose in all species, however, systemic exposure to cidofovir was 2 to 4 fold lower in mice and monkeys compared with rats and rabbits. The half-life ranged from 10 to 30 hours resulting in modest accumulation after multiple administrations. Systemic exposure to CMX064 was highest in monkeys, followed by mice and rabbits, and lastly rats. Recent experiments employing intramuscular dosing produced much higher systemic exposure to CMX001 in monkeys providing rationale for conducting additional efficacy experiments in this species. Human pharmacokinetic studies are ongoing.

1903 OVER-THE-COUNTER ANALGESICS NORMALIZE BLOOD GLUCOSE AND BODY COMPOSITION IN MICE FED A HIGH FAT DIET.

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Type 2 diabetes (non insulin-dependent diabetes mellitus) develops from a pre-diabetic condition that is characterized by insulin resistance and glucose intolerance, and is exacerbated by obesity. In this study, we examined the protection against parameters of a pre-diabetic state in mice fed a high fat diet (6-fold higher in fat than normal chow), by over-the-counter analgesic drugs (OTCAD) [acetaminophen

(APAP); ibuprofen (IBU); naproxen (NAP); aspirin (ASA)]. After 10 weeks on the high fat diet, mice had normal fasting blood glucose (FBG) levels, but exhibited impaired glucose tolerance, which was largely prevented by 20 mg OTCADs/kg body weight, in the order of efficacy, APAP=ASA>IBU, with NAP proving to be ineffective. Mice fed the high fat diet also exhibited increases in weight gain and body fat. Although OTCADs did not prevent weight gain, they prevented in part the increase in body fat, in the order of efficacy, APAP=IBU>NAP=ASA. One protection mechanism for OTCADs is suggested by studies using isolated mitochondria, where OTCADs inhibited the production of reactive oxygen that might otherwise enhance inflammatory processes that would exacerbate the pre-diabetic state. We conclude that OTCADs, especially APAP and IBU, may be valuable tools to help prevent the development of type 2 diabetes from a pre-diabetic condition.

1904 ACETAMINOPHEN NORMALIZES GLUCOSE HOMEOSTASIS IN MOUSE MODELS FOR DIABETES.

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Loss of pancreatic beta cell insulin secretion is the most important element in the progression of type 1 and type 2 diabetes. Since oxidative stress is involved in the progressive loss of beta cell function, we evaluated the potential for the over-the-counter analgesic drug, acetaminophen (APAP), to intervene in the diabetogenic process. We used mouse models for type 1 diabetes (streptozotocin) and type 2 diabetes (high fat diet) to examine the ability of APAP to intervene in the progression of diabetes. In female C57BL/6J mice, streptozotocin caused a dosage dependent increase in fasting blood glucose (FBG), from 100 to ≥600 mg/dl. Daily APAP (20 mg/kg BW, gastric gavage), significantly prevented and partially reversed the increase in FBG levels produced by streptozotocin. After 10 weeks on a high fat diet, mice developed fasting hyperinsulemia and impaired glucose tolerance compared to animals fed a control diet. APAP largely prevented these changes in insulin and glucose tolerance. Furthermore, APAP prevented most of the increase in body fat in mice fed the high fat diet. One protective mechanism for APAP is suggested by studies using isolated liver mitochondria, where low micromolar concentrations abolished the production of reactive oxygen that might otherwise contribute to the destruction of pancreatic beta-cells. These findings suggest that administration of APAP to mice, in a dosage used safely by humans, reduces the production of mitochondrial reactive oxygen and concomitantly prevents the development of type 1 and type 2 diabetes in established animal models.

1905 THE FORMATION OF A QUINONE METHIDE FROM NO-ASA AND ITS ANALOGS CONTRIBUTES TO THE BIOLOGICAL ACTIVITY.

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Nitric oxide donating aspirin (NO-ASA) is a hybrid drug containing a benzyl nitrate linked to aspirin via an ester linkage. The rationale for the development of NO-ASA is to reduce the side effects of aspirin, particularly gastrointestinal toxicity, since aspirin has been identified as a potential chemopreventive agent. However, it is reported that the *para-* and *ortho-* isomers are more potent at inhibiting cell proliferation than the *meta-*. We propose that a short lived quinone methide is formed upon esterase activation, which contributes to the biological activity of NO-ASA. The analogous compounds of NO-ASA, Br-ASA and OMs-ASA, were synthesized to observe their metabolic and biological activities. The isomers of Br-ASA and OMs-ASA were incubated with pig liver esterase and glutathione. In the LC-MS study, the metabolism of the *para-* isomers gives salicylic acid and glutathione adducts of hydromethyl phenol (HMP). Metabolism of the *meta-* mesylate isomer and *meta-* NO-ASA did not generate glutathione adducts, and the *meta-* bromide isomer yielded less glutathione adducts than the *para-* bromide. The formation of glutathione adducts from the more reactive *meta-* Br-ASA is due to a slower S_N2 reaction. *In vitro*, all *para-* isomers induced quinone reductase and the antioxidant response element (ARE) to a greater extent than the *meta-* isomers. Within ten minutes, glutathione depletion in hepG2 cells occurred with the *para-* compounds depleting glutathione more than 25%, with the exception of the slower reacting *para-* OMs-ASA. There was no observed glutathione depletion with the *meta-* isomers. The Comet Assay revealed that the *para-* NO-ASA caused three times more DNA damage than the *meta-* NO-ASA. The results indicate that the quinone methide was exclusively formed from the *para-* and *ortho-* NO-ASA, OMs-ASA, and Br-ASA. The quinone methide formation therefore has an important role in determining the biological activity of NO-ASA, which is not completely due to NO or ASA.

1906 TOPICAL OCULAR MITOMYCIN C TREATMENT IN THE PRESENCE OF A CORNEAL EPITHELIAL DEFECT CAUSED CHANGES IN CORNEAL ENDOTHELIUM.

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Topical application of mitomycin C (MMC) to the eye has been used with increasing frequency in ocular conditions such as conjunctival and corneal intraepithelial neoplasia, and after glaucoma or pytergium surgery and photorefractive keratectomy. Long-term topical use of MMC has been linked to complications such as secondary glaucoma, corneal edema and iritis. Recent in vivo and clinical observations suggest that a combination of exposure to MMC and a compromised epithelium may induce loss of corneal endothelial cells, which are vital to vision. This in vivo study was conducted to evaluate the effect of MMC on corneal endothelial cells in rabbits using a contact specular microscope after corneal epithelium debridement. Baseline specular microscopy was performed on both eyes of the animals. Under general anesthesia, a 10-mm diameter area of corneal epithelium was removed from the cornea of the right eye and 0.02% MMC was applied for 2 minutes, while the left eye was untreated. After the exposure to MMC, both eyes were washed with balanced salt solution. Specular microscopy was performed on both eyes at 7 days after the debridement. The animals were euthanized after 14 days and their corneas were processed using 1% alizarin red staining for microscopic endothelial evaluation. A statistically significant loss of corneal endothelial cells, an increase in endothelial cell size and a decrease in the percentage of hexagonal cell morphology were noted in MMC-treated corneas with specular microscopy. These findings were supported by evaluation of the excised corneas. This study confirms the concern for the topical use of MMC, even short-term, when there is a compromised corneal epithelium. In addition, specular microscopy was demonstrated to be useful in evaluating corneal changes.

1907 THE QUANTITATIVE EVALUATION OF HORMESIS IN ANTICANCER DRUG DOSE-RESPONSES.

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We present here an analysis of the National Cancer Institute's Yeast Anticancer Drug Screen database on dose-response relationships resulting from the evaluation of 2,189 candidate anticancer agents evaluated on 13 yeast (Saccharomyces cerevisiae) strains, resulting in 28,457 replicated dose-responses. A previous analysis of these data has shown that the responses below the toxic threshold were poorly predicted by the threshold dose-response model as defined by the use of Bench Mark Doses (BMDs), and a better fit by a biphasic or hormetic dose-response model. This initial study evaluated the individual responses of concentrations and their replication below estimated BMDs, however it did not explore the individual dose-response relationships. In addition, the initial study did not explore the issue of the magnitude of stimulation with respect to hormesis. This presentation will extend the initial work by exploring both of these limitations. We determined the quantitative features of the hormetic dose-responses (n = 4,548) using a previously published entry and evaluative criteria for inverted U-shaped dose-responses. The quantitative features of the hormetic curves that are described are: (1) the width of the concentration range showing stimulation above 10% of the control (mean of 5.3-fold), (2) the maximum stimulation of the hormetic concentration-responses (mean of 127.4%, compared to a control of 100%), and (3) the width from the maximum stimulation to the zero equivalence point (mean of 6.7-fold). These data are compared across the 13 different yeast genotypes, as well as with previously reported quantitative features of other chemical agents displaying hormetic dose-responses evaluated on various endpoints and model organisms. These data suggest that more than half (1,125 of 2,189) of the anticancer agents evaluated display hormetic dose-responses in the 13 NCI yeast strains used as models for human cancers.

1908 4-ANDROSTEN-3, 6, 17-TRIONE (6-OXO™) DOES NOT HAVE ANDROGENIC OR ANABOLIC ACTIVITY IN HERSHBERGER ASSAY.

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The androgenic and anabolic effects of 4-Androsten-3, 6, 17-trione (6-OXO™), a new dietary supplement, were studied by Hershberger assay in CD® rats. Castrated male rats (six per dose group) were administered, by subcutaneous injection, 6-OXO™ at doses of 0, 0.1 and 2.0 mg/kg/day or an androgen agonist, testosterone propionate, at a dose of 1.6 mg/kg/day for 10 consecutive days. HPLC methods of

analyses of 6-OXO™ and testosterone propionate were developed and validated. All dosage formulations were within ± 10% of their respective target concentrations. No clinical signs of toxicity, changes in body weights or organ weights were seen in any dose group administered 6-OXOTM when compared to the control group. In the testosterone propionate-treated group, body weight gains (on day 4), total weight gains and all measured organ weights (ventral prostate, levator ani plus bulbocavernosus muscles, and seminal vesicles with coagulating glands) were significantly increased when compared to the control group. These data suggest that 6-OXO™ does not possess the androgenic and anabolic activity under conditions of this study.

1909 PWT-458 RANGE FINDING STUDY: COMPARISON OF ORAL AND INTRAVENOUS TOXICITY AFTER ONE WEEK OF DOSING IN RATS.

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PWT-458 dosed intravenously (IV) was previously associated with cardiac necrosis in rats at dosages of 150 mg/kg/day or 300 mg/kg/day. In this study, we investigated the potential toxicity of oral dosing of PWT-458 compared with IV dosing in rats. Groups of 8 CD IGS Sprague-Dawley rats were dosed daily for seven days with PWT-458 orally at 50, 150, or 300 mg/kg/day, or IV at 150 mg/kg. Four of the 8 rats dosed orally with 300 mg/kg/day did not survive to study termination. The cause of death was related to necrosis in multiple tissues. Rats administered PWT-458 at 150 or 300 mg/kg/day orally lost weight during the study period in a dose-related manner. Body weight gain was suppressed in rats administered PWT-458 at 50 mg/kg/day orally compared with weight gain in vehicle-dosed rats during the study period. Microscopically, a number of findings were observed only after oral dosing. Rats dosed orally had test article related cardiac necrosis at 150 or 300 mg/kg/day. Additionally, the 300 mg/kg/day group had foamy macrophage infiltration in the heart. Renal tubular necrosis was present in 6 of 8 rats dosed orally at 300 mg/kg/day. Rats dosed orally also had duodenal and jejunal tissue necrosis and atrophy, and gut associated lymphoid tissue necrosis and atrophy, with an incidence of 2 to 7 of 8 rats at 150 or 300 mg/kg/day, respectively. The group dosed orally with 300 mg/kg/day of PWT-458 also had gastric mucosal necrosis in 5 of 8 rats. None of these findings were present after IV dosing. Test article related splenic atrophy was present in both orally and intravenously dosed rats with an equal incidence of 7 of 8 rats at all dose levels. Renal tubular ectasia was present in 3, 2, and 5 of 8 rats dosed orally with 50, 150, or 300 mg/kg/day, respectively, and in 1 of 8 rats dosed IV at 150 mg/kg/day. In summary, administration of PWT-458 by the oral route resulted in significant toxicity that was more severe overall compared with the IV route.

1910 NOVEL ASPECTS OF ACQUIRED CANCER CELL CHEMO- AND RADIO-RESISTANCE.

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The successful treatment of cancer requires a clear understanding of multiple interacting factors involved in treatment resistance. Presently, two hypotheses, genetic and epigenetic, have been proposed to explain mechanisms of acquired cancer treatment resistance. The absence of convincing evidence that genetic changes have a role in acquired clinical resistance undermines the genetic hypothesis. In contrast, a number of studies indicate a substantial involvement of epigenetic mechanisms in drug-resistant cancer cells, including changes in DNA methylation and histone modification patterns.

In the present study, we first examined the epigenetic changes in MCF-7 human breast cancer cells resistant to doxorubicin (MCF-7/DOX) and cisplatin (MCF-7/cisDDP). The MCF-7/DOX cells are characterized by increased expression of P-glycoprotein, whereas MCF-7/cisDDP cells are characterized by increased expression of GSTπ. Both of these drug-resistant cell lines displayed similar pronounced changes in global and locus-specific epigenetic landscape showing loss of global DNA methylation, loss of histone H4K20 trimethylation, and diminished expression of Suv4-20h2 histone methyltransferase compared to parental MCF-7 cells. Ionizing radiation is another important treatment modality. Therefore, the next step of our study was aimed to analyze and compare the resistance of the MCF-7, MCF-7/DOX and MCF-7/cisDDP cells to radiation. We found that chemoresistant cells are able to withstand killing by radiation. MCF-7/DOX and MCF-7/cisDDP cells do not undergo radiation-induced cell cycle arrest and apoptosis. Additionally, chemoresistant cells have altered capacity to repair radiation-induced damage.

These results provide the evidence that epigenetic changes are an important feature of cancer cells with acquired drug-resistant phenotype. Furthermore, deregulation of similar pathways may explain the existence and provide mechanism of cross-resistance of cancer cells to different types of chemotherapeutic agents and to radiotherapy.

1911 PRECLINICAL EVALUATION OF ACUTE TOXICITY OF DECITABINE (5-AZA-2'-DEOXYCYTIDINE).

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The acute toxicity of decitabine (5-Aza-2'-deoxycytidine), an experimental agent for sickle cell disease treatment, was investigated in CD® rats and Beagle dogs. Rats (five per sex/dose group) were administered a single oral gavage at doses of 0, 100, 500 and 2000 mg/kg. There were a treatment-related decreases in body weight gains on days 1-8 of observation in the 2000 mg/kg dose group with recovery occurring on day 15. The changes of spleen and thymus relative weights (more pronounced in males) were observed only in the 2000 mg/kg dose group. Decitabine at a dose of 2000 mg/kg produced overt toxicity in male rats without mortality and can be considered as MTD (maximum tolerated dose). The decitabine administration at a dose of 100 mg/kg produced mild reticulocytopenia and leucopenia consistent to the drug pharmacologic effect. Partial recovery of hematology parameters was seen on day 10. The 100 mg/kg dose was considered below toxicity levels. Dogs (one per sex/dose group) were administered once orally by capsule at doses of 0.4, 2 and 10 mg/kg. Decreased activity, lethargy, dehydration, diarrhea and loss of appetite were observed in the 10 mg/kg dose group. Dose-dependent decreases in body weight were seen in all dogs. On day 15 after dosing, a hepatotoxicity in the 10 mg/kg dose group was observed (changes in cholesterol, alkaline phosphatase, albumin, globulin, triglicerides levels, and total protein and serum inorganic phosphorus concentrations). Anemia, reticulocytopenia, leukopenia and changes in coagulation parameters were seen in the 10 and 2 mg/kg dose groups. A slight leukopenia with recovery on day 15 was seen in the 0.4 mg/kg dose group. Decitabine at 10 mg/kg produced severe toxicity in dogs. The dose of 2 mg/kg can be considered as MTD. Under these experimental conditions, decitabine produced marked hematopoietic toxicity and hepatotoxicity that were much more pronounced in dogs.

1912 ENDOTOXIN ENHANCES ACETAMINOPHEN-INDUCED NASAL TOXICITY IN MICE.

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Acetaminophen (APAP) is a leading cause of liver injury in people and also causes toxicity to the liver, lung, nose and kidneys in mice at high doses. Previously, we have reported that cotreatment of mice with nonhepatotoxic doses of lipopolysaccharide (LPS) and APAP results in hepatotoxicity, as evidenced by increased plasma alanine aminotransferase (ALT) activity. The purpose of the present study was to determine if a similar cotreatment of LPS and APAP causes nasal toxicity in mice. Fasted, C57BL/J6 mice were treated with either saline or LPS (4.4 x 107 EU/kg, i.p.) two hours before treatment with a nonhepatotoxic dose of APAP (150 mg/kg, i.p.) or saline alone (controls). Other mice received only a hepatotoxic dose of APAP (300 mg/kg). Mice were sacrificed twenty-four hours after APAP treatment. Plasma was collected for ALT activity analysis, and nasal tissues were processed for morphometric analysis. Plasma ALT activity was normal in mice exposed to either LPS or 150 mg/kg APAP alone but was increased in mice given 300 mg/kg APAP or the combination of LPS plus 150 mg/kg APAP. No nasal epithelial lesions were present in mice treated with LPS or APAP 150 mg/kg alone. Minimal nasal epithelial injury was evident in mice given the high dose of APAP alone. Mice that received both LPS and 150 mg/kg APAP had marked degeneration, necrosis and exfoliation of epithelial cells lining the proximal nasal airways, resulting in a marked decrease (31% lower than controls) in epithelial cell density. These mice also had a mild neutrophilic rhinitis. These results demonstrated a synergistic interaction between APAP and LPS that caused marked nasal toxicity in mice. The mechanisms underlying this target organ toxicity is yet unknown. (Supported in part by the MSU Respiratory Research Initiative)

1913 SYNERGISTIC TOXIC EFFECTS OF ACETAMINOPHEN AND OZONE ON MURINE NASAL EPITHELIUM.

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Acetaminophen (APAP), a leading cause of liver injury in people, is known to induce, at high doses, hepatic, pulmonary, nasal and renal toxicity in laboratory rodents. APAP toxicity is associated with P450-mediated generation of N-acetyl-p-benzoquinone imine, which causes glutathione depletion and adduction. Acute exposure of ozone (O3), the principal oxidant pollutant in photochemical smog, may also lower glutathione levels and cause toxicity in murine airways. The purpose of the present study was to test the hypothesis that APAP and O3 may act synergistically to cause nasal toxicity. Eight-week-old, C57/BL6 male mice were fasted

overnight and then treated with APAP in saline (150 or 300 mg/kg i.p). Two-hours later, mice were exposed to 0, 0.25, or 0.5 ppm O3 for 6h. Mice were sacrificed twenty-four hours after O3 exposure. Plasma was collected for alanine aminotransferase (ALT) analysis, an indicator of hepatotoxicity, and nasal and liver tissues were processed for light microscopic examination and morphometric analysis. APAP alone caused an increase in plasma ALT activity at the high but not the low dose. O3 alone had no effect on ALT activity. No nasal toxicity was present in mice that received O3 or APAP alone. In contrast, mice that received both 300 mg/kg APAP and 0.25 or 0.5 ppm O3 had a significant decrease (46-50% less than controls) in nasal epithelial cell density due to marked degeneration, necrosis and exfoliation. These results demonstrated that coexposures of O3 and APAP, at doses that given alone cause no or minimal nasal toxicity, induced a synergistic toxic effect on the murine nasal epithelium. The underlying mechanisms are yet to be determined. (Supported in part by the MSU Respiratory Research Initiative)

1914 AH RECEPTOR – AN UNKNOWN TARGET OF IMMUNO-MODULATORY DRUGS?

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Dioxins are potent mammalian toxins that affect cellular proliferation and differentiation. The aryl hydrocarbon receptor (AhR) is a ligand activated transcription factor belonging to the basic-helix-loop-helix (bHLH)-Per-AhR/Arnt-Sim (PAS) protein family and mediates the toxicity of dioxins and related chemicals. During a search for molecules that activate AhR, Leflunomide®, an immunomodulatory drug used to treat a number of diseases, was identified to potently activate AhR transcription. A known mechanism of action of Leflunomide is through one of its metabolites, A771726, which inhibits de novo pyrimidine synthesis. Interestingly, CYP1A2, an AhR inducible gene has been implicated in the metabolism of Leflunomide to A771726. Leflunomide, but not A771726, activated AhR transcriptional activity in cell based reporter assays, and up-regulated a number of genes (CYP1A1, CYP1A2, UDP-glycosyl transferase, and NADPH quinone oxidoreductase) strictly in an AhR-dependent manner. Proliferation of T cells expressing AhR was inhibited by exposure to Leflunomide. Studies are in progress to determine the requirement of AhR in mediating Leflunomide-induced inhibition in proliferation. AhR may mediate biological activity of Leflunomide by two different means (i) by controlling the metabolism of Leflunomide and/or (ii) by directly mediating the effects of Leflunomide. It is well known that AhR mediates dioxin-induced immune suppression. This raises the intriguing possibility of targeting AhR for controlling hyperimmune disorders.

1915 NONCLINICAL TOXICOLOGY PROFILE OF AMG 517, A NOVEL TRPV1 ANTAGONIST CONSIDERED FOR CLINICAL USE AS A PAIN THERAPEUTIC.

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The vanilloid receptor TRPV1, is a membrane bound non-selective cation channel with high permeability to calcium, which is expressed in peripheral sensory neurons. AMG 517 is a small-molecule competitive antagonist at TRPV1 that blocks activation of the channel. Accordingly, AMG 517 was considered for clinical use in the treatment of pain. A series of nonclinical toxicology studies was conducted with AMG 517 to support early clinical development. These studies were conducted in multiple species and included single and repeated-dose studies (up to 28 days in duration), genotoxicity studies, safety pharmacology studies and assorted special toxicity studies.

AMG 517 was not genotoxic and did not significantly inhibit hERG tail current in vitro. However, low incidence of ECG changes of uncertain significance and unlikely relationship to drug-treatment were observed in 2 single-dose oral studies conducted in cynomolgus monkeys. The other in vivo safety studies indicated that AMG 517 was well tolerated. However, AMG 517 caused transient increases in body temperature in all evaluated species that lasted up to ~24 hours. The highest body temperatures generally occurred within a few hours of dosing and declined thereafter. The hyperthermic effect appeared to attenuate with repeated dosing, as the magnitude of effect was typically less pronounced after the first day of dosing. Due to the absence of associated untoward observations and their transient nature, the hyperthermic observations were not believed to be adverse in nonclinical models. However, based on observations with AMG 517 and other TRPV1 antagonists, and the fact that treatment with agonists (e.g. capsaicin) is known to produce hypothermia, the findings were believed to represent a pharmacological effect of TRPV1 antagonism.

Based on the results from the comprehensive nonclinical toxicology package, the program was deemed adequate to support phase I trials of AMG 517 with safety monitoring of findings identified in the nonclinical studies including ECGs, and body temperature.

1916 A PANEL OF LIVER SPECIFIC PROTEINS AS BLOOD BIOMARKERS FOR DRUG INDUCED LIVER INJURY.

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Drug induced liver injury (DILI) is a potential complication of nearly every medication that is prescribed, however, the current liver enzyme assay used to monitor liver injury has limitations since elevated enzyme levels may or may not be a signal of serious liver damage. While a single biomarker is unlikely to accurately indicate DILI, characteristic signatures obtained with a panel of biomarkers may be more indicative of the severity of the liver injury. The purpose of the current study was to measure and identify changes in liver-specific (or relatively specific) proteins in the serum during early toxic effects, maximal toxic effects, and the recovery stages of liver injury. Through the analysis of our human and mouse transcriptome database (multiple parallel signature sequences or MPSS), we identified 133 liver-specific genes. We purchased 119 commercially available antibodies for 65 liver specific proteins. In collaboration with Aviva Systems Biology, we generated rabbit polyclonal antibodies for 75 liver specific proteins with multiple epitopes. Three mouse strains were administered 300 mg/kg acetaminophen by i.p. injection. Subsequent histopathological exams showed different levels of severity of liver injury in the three different mice strains (C57BL/6J>A/J>SJL). Mouse blood was drawn from acetaminophen treated or control mice at 3h, 12h, 24h, 48h, and 96h post-injection. We established a high-throughput, label-free method for analyzing serum using Surface Plasmon Resonance (SPR) imaging of antibody microarrays (Lumera Nanocapture Gold™). Relative expression profiles were generated, recorded and compared with the histopathology data. Hierarchical clustering of the serum protein levels revealed a distinct hepatotoxic profile corresponding to different levels of severity of liver damage. This antibody chip can be widely used for diagnostics applications and by the pharmaceutical industry.

1917 METABOLOMICS: URINARY MARKERS OF DRUG-INDUCED LIVER INJURY WITH CORRELATION WITH LOBE VARIATIONS IN RESPONSE.

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This study was conducted to evaluate the use of metabolomics for identifying biomarkers of drug-induced liver injury and associate these markers with specific biochemical pathways to give insight into the mechanism of liver injury. Rats were dosed with vehicle, no effect levels, or effect levels of clofibrate, valproic acid, isoniazid, phenytoin, or acetaminophen: the latter four drugs being the focus of the NIDDK clinical trials for drug-induced liver injury (DILI) and Idiosyncratic Liver Injury (ILIAD). Urine was collected for 24 h (on dry ice using all glass metabolism chambers) from animals after 1 day of dosing or for up to 14 days of dosing. Liver was processed for examination of pathology for the right, left, and median lobes. The remaining liver was extracted for metabolomic analysis. Serum enzyme levels were also measured. Metabolomics of urine collected from rats exposed to vehicle, no effect doses, or effect doses exhibited different patterns for endogenous metabolites following 1 day or multiple days of dosing. Sub-sets of metabolites could be associated with specific pathology, while serum enzyme levels often failed to correlate with pathology outcomes. Metabolomic profiles of liver extracts were different between vehicle and dose groups, and profiles could be used to differentiate liver lobes. The relevance of metabolites that are significant to the separation of groups based on dose or pathology are being determined through pathway mapping. The application of metabolomics to develop urine based markers that can be used to screen, as an early marker, for drug-induced liver injury and linking markers that are reflective of specific mechanisms of liver injury to biochemical pathways will help gain a mechanistic understanding of the on-set or recovery from liver-injury. This study is funded by NIGMS grant 5R21GM075903.

1918 HEPATIC SIDE EFFECTS, IDIOSYNCRATIC OR TOXIC MECHANISMS – DIFFERENTIATED EVALUATION FOR REGULATORY AFFAIRS FOR HERBAL REMEDIES.

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In the last decade also for herbal remedies side effects and interactions with other remedies are reported. Unfortunately these observations by herbal remedies are evaluated often without a necessary differentiation by a general prescription of the drug. On three recent occasions in Germany drug regulatory decisions for herbal drugs (Meliloti herba, Piperis methystici rhizoma, Chelidonii herba) have been based on possible hepatotoxicity as suspected adverse effects. These decisions have been contested on the ground that adverse effect attributions have not been based on scientific evidence but mainly on animal experimental data and time coinci-

dence. Whereas toxic liver damage may be limited by intake limitation, restricting daily intake is no preventive strategy for idiosyncratic reactions. Whether or not regulatory actions should be taken ought to be based on proven (or likely) causal association indicated by the parameters given above; also the availability of safer therapeutic alternatives should be considered in a benefit risk analysis. Additionally combinations with critical drugs should be considered independently, because the other herbal drugs can protect and reduce possible side effects. On that account before a proven herbal remedy is cancelled because of supposed critical hepatic side effects the following needs to be clarified: the level of the background-rate of liver injuries based on the disease itself has to be checked. Also it has to be proven, if there are therapy alternatives with lower side effects or if there are other problems at the alternatives (drug-dependence by benzodiazepines). In general the patients need better information in the leaflets to alcohol abuse or interaction caused on primary liver diseases.

1919 MULTI-SITE TECHNICAL VALIDATION OF NOVEL PRECLINICAL SERUM ENZYME MARKERS OF HEPATOTOXICITY.

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Acceptance of new biomarkers requires rigorous characterization of the assay's technical attributes, followed by biological qualification of the analyte in the context of the applications of interest. In an effort to expedite and standardize the technical validation of four candidate serum enzyme markers for liver injury in rats, three participant laboratories in the Hepatotoxicity Working Group (HWG) of Critical Path Institute's Predictive Safety Testing Consortium (PSTC) conducted collaborative cross-site testing. Commercial assays were identified for glutamate dehydrogenase and common analytical protocols were implemented for paraoxonase-1, purine nucleoside phosphorylase, and malate dehydrogenase. Two brands and three models of automated chemistry analyzers were represented across the sites. Each lab evaluated the following performance characteristics for each analyte according to harmonized guidelines: intra- and inter-assay precision, linearity, lower limits of quantitation, lower limits of detection, and recovery. Reference ranges for healthy rats were determined by each site. Performance across sites was conducted by exchange of identical control materials, split samples from healthy rats, and split samples from animals with liver histopathologic changes classified according to a standardized PSTC lexicon. Analyte stability and potential interference were assessed by one site. Select pre-analytical variables were also examined by at least one site, e.g., rat strain (Sprague-Dawley, Wistar), gender, and sample type (serum, plasma). As a result of this successful HWG collaboration, subsequent biological qualification of these biomarker nominees can be accelerated with preclinical samples submitted by participant companies across a range of hepatotoxicities.

1920 PREDICTIVE AND MECHANISTIC TOXICOLOGY OF NANOPARTICULATE CARRIER BY HEPATIC GENE EXPRESSION PROFILING IN THE RAT.

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The comparison of gene expression profiles with drug toxicity signature databases is a powerful tool in predictive and mechanistic toxicology. We report here the effects on rat hepatic gene transcription of treatment with a new polymer which self-assembles to form poly-amino acid nanoparticles. This new polymer is a versatile carrier for biologically active molecules without pharmacological action when administered alone. Male Sprague-Dawley rats were treated by a single intravenous injection at 2.5, 100 or 200 mg/kg on Day 1. Liver samples were taken from animals sacrificed 4 hours or 2 and 6 days after dosing. Liver transcriptional profiles were studied using Affymetrix Genechip® Rat Genome 230 2.0 arrays. In addition to a classical data analysis, data were sent to Iconix's DrugMatrix™ signature database using the ToxFX analysis suite.
Treatment with the test item was clinically well tolerated, without test item-related clinical signs or major effects on body weight.
Four hours after treatment at 200 mg/kg, transcriptional profile changes in the liver were concordant with a hepatic inflammatory infiltrate, based on the gene ontology classes which were affected and the strong matches to inflammation related drug toxicity signatures.
Two days after treatment, the high dose-level produced changes in the transcription of genes involved in cell proliferation and cytoskeleton reorganization. Comparison with the DrugMatrix™ database gave strong matches with hypoalbuminemia and serum alanine aminotransferase increase signatures.
At these two time-points, the effects at 100 mg/kg were dose related and less pronounced.
The changes in the hepatic gene transcription were concordant with non specific Kupffer cell activation induced by the test item nanoparticle phagocytosis. These minimal effects were reversible and transitory, with a return to basal expression levels 6 days after dosing.
Administration of the human equivalent clinical dose (2.5 mg/kg) did not induce any significant gene transcription changes in the liver.

1921 HEPATIC TRANSCRIPTIONAL PROFILING AS AN INDICATOR OF INFLAMMATORY/ACUTE-PHASE RESPONSES.

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Liver tissue may be an underutilized source for the detection of inflammatory responses to drugs and toxicants in drug-safety studies. This is highlighted by the critical role of the liver in the synthesis of acute phase proteins, complement factors, cytokines, and interleukins. We hypothesized that hepatic transcriptional changes would provide a useful assessment of inflammatory status in rats including the ability to detect extra-hepatic inflammation and alterations missed by traditional endpoints. To examine this hypothesis, transcriptional changes mediated by approximately 40 novel therapeutic compounds administered to rats for 3 days to 3 weeks were evaluated. Compounds examined produced extra-hepatic inflammation, modulated inflammatory responses, or did not produce inflammation. Livers from drug- and vehicle-treated animals were analyzed by microarray for evidence of modulation of inflammatory transcripts. Evaluation included analysis of treatment-altered transcripts ($P<0.01$) within Gene Ontology (GO) categories and correlation of GO hits with findings from traditional pathology endpoints. GO category results were considered positive if $P<0.01$. Recurring positive GO category results included "inflammatory response", "immune response", "complement activation", and "acute phase". Significant transcriptional changes in inflammation-related GO pathways were observed in rat liver following treatments causing extra-hepatic and hepatic inflammation. Treatments causing inflammation and altering inflammation-related clinical pathology parameters also generally altered inflammation-related transcripts in the liver robustly. Also observed was a repression of inflammation-related transcripts in treatments producing generalized toxicity. These data suggest that rat hepatic transcriptional profiling provides a useful assessment of inflammatory state by capturing pharmacologic effects undetected by traditional end points and provides an additional marker for hepatic and extra-hepatic inflammatory pathology.

1922 DRUG-INDUCED LIPIDOSIS IN THE DOG GALLBLADDER: A MECHANISTIC STUDY COMBINING CONVENTIONAL AND METABONOMIC TOOLS.

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As part of its early preclinical development, a new drug candidate induced gallbladder lesions after 1 to 4 weeks of oral dosing in dogs. They were characterized by unusual fat vacuolation in the gallbladder epithelium and in submucosal macrophages, positive for Oil Red-O staining. This was associated with increased epithelial mitoses/apoptoses, epithelial lipofuscin pigment accumulation and inflammatory cell infiltration in the lamina propria. This toxicity was clinically silent, no lesions were observed in the liver or biliary tract and conventional clinical pathology did not evidence any surrogate biomarker. A mechanistic study was then performed on biliary cannulated dogs dosed for 2 weeks at a toxic dose with sequential collections of bile in pretest and at 1, 7 and 14 days of dosing. Specific bile analysis included search for mucins and bile crystals. In parallel, a metabonomic approach based on UPLC-MS (Tof and Qtrap) analysis was applied for both the determination of bile composition (biliary acids and lipids) and for drug metabolite identifications. Numerous metabolites, as well as the drug itself, were identified in the dog bile, suggesting that the observed toxicity could be exerted via a concentration in the gallbladder of exogenous molecules. Bile endogenous metabolite patterns were clearly different between pretest and dosing periods, with major changes at 1 and 7 days. Bile acids or pigments were unchanged and bile mucins were inconsistently increased or decreased. The main biliary modification was a lipid imbalance with a decrease in several triglycerides, a decrease in free cholesterol with a parallel increase in phospholipids, thus a modified cholesterol to phospholipids ratio. This could reflect an increased gallbladder epithelium absorption of some neutral lipid components from the bile and their subsequent epithelial accumulation, revealed by the Oil Red-O positive stain. These results show how conventional and more recent metabonomic tools can be combined in studying mechanisms of toxicity.

1923 THE USE OF PRECISION-CUT LIVER SLICES TO ASSESS THE SPECIES SPECIFIC TOXICITY OF KINASE INHIBITORS.

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Dog specific-sensitivity of small molecule kinase inhibitors is widely known in the pharmaceutical industry. These inhibitors have also been known to cause adverse liver toxicity in man due to their wide ranging role in cell cycle and cytokine signal transduction. There are currently no reliable in vivo tools available that can assess species-specific toxicity of kinase inhibitors. Therefore, we used in vitro precision-cut tissue slices as a model to assess the species variation in toxicity of a kinase inhibitor (ABC123), currently in development by Novartis, in rat, dog and human liver slices. Two separate preparations of rat, dog and human liver slices were treated with ABC123 for 24 and 48 hours at concentrations of 0, 50, 100, 250 and 500 µM. At each time point the clinically relevant liver toxicity marker AST and ALT as well as LDH leakages were used to determine membrane integrity and cell damage. ABC123 showed species differences in terms of toxicity in rat, dog and human liver slices. In rat liver slices no toxic effects were observed with ABC123 treatment at any concentration or time point tested. One human liver slice preparation showed a marginal, but significant increase in LDH and ALT levels at 48 hrs with 500 µM ABC123. Dog liver slices showed a clear concentration- and time-dependent increase in all leakage markers. More specifically, 250 µM ABC123 was not toxic in human and rat liver slices after 24 and 48 hrs. The same concentration of ABC123 in one dog liver slice preparation resulted in significant increase in LDH and ALT release after 24 hrs and all three leakage markers were increased markedly after 48 hrs in both preparations. Based on these data, dog liver slices were found to be more sensitive to ABC123 treatment as compared to human and rat slices. This study demonstrates that precision-cut tissue slices can be effectively employed to evaluate species variation in vitro.

1924 A 28-DAY TOXICITY STUDY OF KRX-0502 (FERRIC CITRATE) IN RATS BY DIETARY ADMINISTRATION.

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When administered orally, KRX-0502 (ferric citrate) binds inorganic phosphate in the GI tract and prevents phosphate absorption. KRX-0502 is therefore being developed for the treatment of hyperphosphatemia in patients with end stage renal disease (ESRD). In a dose-ranging clinical study, KRX-0502 was shown to significantly lower serum phosphate concentrations when administered to ESRD patients at 6 g/day (Niecestro et al 2007 World Congress of Nephrology). Although ferric citrate is Generally Regarded as Safe (GRAS) when used as a food supplement, additional studies are needed to support further development and to evaluate toxicity at the substantially higher doses required to prevent phosphate absorption from the GI tract. In a 28-day toxicity study in Sprague-Dawley rats, conducted to evaluate KRX-0502 toxicity, 5 animals/sex/dose were administered 0 (vehicle), 500, 2000 or 3500 mg/kg/day KRX-0502 in standard rodent diet. Assessments included clinical signs, food consumption, body weight, clinical laboratory, gross necropsy and selected histopathology. There were no deaths or clinical signs or changes in serum iron parameters. After 4 weeks, mean body weights in both sexes were decreased by 5% and 8% at 2000 and 3500 mg/kg/day, respectively. Urinary excretion of phosphorus at 2000 and 3500 mg/kg/day was decreased 79% to 98%, consistent with lower phosphorus absorption, but there was an increase in serum phosphorus at these doses in males (24% and 33%, respectively) and females (20% and 37%, respectively). Since urinary calcium excretion was increased 3.5 to 14.5-fold at 2000 and 3500 mg/kg/day, an increase in bone resorption was considered the source of the higher serum phosphorus levels. However, an increase in bone resorption was not detected under the conditions of this study by microscopic evaluation or serum PTH levels. The No Observed Effect Level was 500 mg/kg/day, and the No Observed Adverse Effect level was 3500 mg/kg/day since this dose was clinically well tolerated.

1925 EARLY GENTAMICIN TOXICITY URINE BIOMARKER IDENTIFICATION IN RATS USING AN NMR CHEMOMETRIC AND METABOLOMIC APPROACH.

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Gentamicin is an aminoglycoside antibiotic prescribed to treat life-threatening infections. Treatment with gentamicin is associated with a 10-15% incidence of nephrotoxicity, which is revealed as an increase in serum creatinine or acute renal failure. In addition, animal models demonstrate acute tubular necrosis, which may be the cause of a known clinical reduction in glomerular filtration rate. In order to elucidate specific biomarkers of gentamicin toxicity, urine was collected from 25, 40, and 80 day-old male Sprague-Dawley rats (n=4 animals per dose group) dosed with saline, 50, or 100 mg/kg of gentamicin for six days at 0, 8, 24, 48 and 72 hours during the dosing period. ^{1}H nuclear magnetic resonance spectroscopy has been shown to be a highly quantitative and reproducible tool for the analytical characterization of biofluids, and as such was employed for metabolite analysis. In addition, kidney pathology was assessed at sacrifice, 24 hrs after the last dose was administered (168 hours) for each animal. The urine NMR spectra and pathology scores were chemometrically compared using a supervised orthogonal partial least squares (OPLS) modeling approach. Candidate biomarkers were identified from the most influential spectral regions using a statistical correlation approach and subsequent comparision with pure reference spectra from the Chenomx database. Hippurate was the most dramatic biomarker identified known to be an indicator of gentamicin antibiotic activity due to its impact on gut-microflora content. In addition, concentrations of allantoin, creatinine, and N,N-dimethylglycine were shown to be increased in a manner consistent with the pathology results. Importantly, the metabolomics analysis was able to detect toxicity-related markers based on 72 hour urine, much earlier than traditional clinical chemistry tests.

1926 URINE COLLECTION AND ANALYSIS IN REGULATORY TOXICOLOGY STUDIES: A CROSS-LABORATORY COMPARISON.

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Regulatory toxicology studies for pharmaceutical development are well defined in terms of purpose, design and types of data collected. LASA and NC3Rs established a Safety Evaluation Working Group of toxicologists from UK major pharmaceutical companies and contract research organisations, sharing best practice and identifying areas for application of the 3Rs whilst keeping scientific integrity and regulatory compliance of studies. An early initiative reviewed urine collection and analysis, focussing on opportunities for reduction and refinement. Urine collection can be considered non-invasive, but isolation in a metabolism cage in a barren environment separated from cage mates without food and sometimes water is stressful for animals. For larger species, catheterisation also has associated welfare issues. The working group approached UK clinical pathologists to identify cases where toxicity was seen from urine analysis that was not evident in another study parameter, eg plasma clinical chemistry, histopathology. Data were collected from each laboratory on the methods for urine collection and frequency of urine collection for mouse, rat, dog and non-human primate. Examples showing the value of urine analysis alone were rare. The quality of urine samples was variable and their value, on occasion, limited. There were variations in methods for all species in 1) time animals spend in a metabolism cage, 2) use of catheters, 3) food and/or water deprivation and 4) frequency of collection of urine samples. Further work is necessary to establish the specific scientific rationale for urine analysis to either 1) challenge the requirement/frequency for urine analysis or 2) improve quality of urine collection methods. The scientific rationale will be critical to recommendations on refinement and reduction from an animal welfare perspective. For NC3Rs/LASA Safety Evaluation Working Group

1927 METABONOMIC ANALYSIS OF URINE IN CISPLATIN-INDUCED RENAL TOXICITY IN RATS AND MICE.

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Experimental means to assess drug toxicity in pediatric populations are limited. This study utilized a multi-age rat model to examine potential age-dependent differences in drug-induced renal toxicity. Male Sprague-Dawley rats (25, 40, 80 days old) were injected with a single dose of cisplatin (cip) (0, 1, 3 or 6 mg/kg i.p.). Blood and tissues were obtained at necropsy and histopatholgy scores were recorded. Urine samples were collected at 0, 24, 48 and 72 hrs after the treatment. All animals given high dose cip lost weight and kidney weight/body weight ratios only significantly decreased in 80-day-old rats. Similarly, 80-, and 40-day-old rats had moderate to severe renal lesions (acute tubular necrosis and apoptosis) and significantly increased BUN and serum creatinine levels. In a second study, four 8- to 10-week old Sv129 mice received a single dose (20 mg/kg BW) of cisplatin and urine samples were collected on days 0, 1, 2, and 3 after cip dosing. NMR and MS metabolomic methods were used in both studies to detect biomarkers that could

predict nephrotoxicity. In the mice cip study there were increased urinary levels of hydroxyproline and glucose at day 2 after cisplatin injection which precedes the rise in BUN and serum creatinine not seen until after 3 days of cip injection. In the rat studies, glucosuria and hydroxyprolinuria were also observed on day 2 and 3 in 40- and 80-day old rats which preceded the BUN and creatinine increases detected at 72 hours. These results indicate that the extent of cisplatin-induced renal toxicity is dependent on age and that certain translational metabolic biomarkers can help identify cisplatin induced renal toxicity. Increased kidney epithelial cell extracellular matrix degradation and more specifically collagen degradation by cisplatin mediated activation of kidney metalloproteases could explain the observed increased in urinary hydroxyproline.

1928 PRECLINICAL EVALUATION OF IMMUNOMODULATORY ACTIVITY AND SAFETY OF RECOMBINANT HUMAN INTERLEUKIN-21.

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Interleukin-21 (IL-21) is a type I cytokine produced by activated CD4+ T cells that affects both innate and adaptive immunity. Effects of IL-21 include activation, increased proliferation, and prolonged survival of CD8+ T cells; enhancement of T cell dependent B cell proliferation and antibody production; and terminal differentiation and activation of NK cells. These activities are believed to contribute to the antitumor activity of recombinant IL 21 (rIL-21) observed in preclinical models and early clinical trials. The non-clinical safety profile of rIL-21 was evaluated in cynomolgus and rhesus monkeys. A broad dose range, from 0.01 to 3 mg/kg of rIL-21 administered intravenously (IV) was studied using an intended clinical dose regimen of two or four treatment cycles consisting of five consecutive daily IV doses followed by nine dose-free days. Hematological effects, most notably decreased erythrocyte mass and platelet count constituted the primary toxicological effects of rIL-21. These effects were present at doses of 0.1 mg/kg, were dose limiting at ≥ 0.5 mg/kg, and were reversible during the non dosing intervals. Transient increases in body temperature and changes in serum proteins indicative of an acute phase response (i.e., increased C-reactive protein) were observed. Transient decreases in circulating lymphocytes were observed, followed by rebound lymphocytosis. Increased serum soluble CD25, a pharmacologic marker for IL-21 action on lymphocytes, was also observed. Consistent with the expected pharmacology for IL-21, tissue microscopy demonstrated decreased size and cellularity of germinal centers (B cell region), increased numbers of plasma cells, and decreased cellularity within T cell regions in spleens and lymph nodes. Under the conditions tested, rIL-21 had pharmacological activity consistent with its immunostimulation mechanism of action. The toxicities observed were reversible and monitorable in clinical trials and these data formed the basis of support for initial clinical testing of IL-21 in cancer patients.

1929 INDUCTION OF IMMUNE TOLERANCE TO AMPICILLIN FOLLOWING ORAL DOSING IN SPRAGUE-DAWLEY RATS.

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Drug allergies are among the most common clinical adverse events associated with drug treatment. Recently it has been hypothesized that immune tolerance, rather than sensitization, may be induced in a majority of individuals that are administered antigenic drugs by the oral route, and a lack of immune tolerance may in part contribute to the idiosyncratic nature of allergic reactions in humans. To test this hypothesis and begin to elucidate the mechanism of tolerance to orally administered drugs, ampicillin (AMP) was selected as a tool compound. Sprague-Dawley rats were orally pretreated with vehicle, 30, 100 or 300 mg/kg AMP, or 50 mg/kg oxazolone (OXZ) as a positive control for 14 days. Following an off-drug period of 17 days, the rats were given three daily subcutaneous (SC) challenge doses (100% and 1%, AMP and OXZ, respectively). Tolerance was evaluated by attenuation of BrdU incorporation (cell proliferation) in lymphocytes in the draining lymph node. In vehicle pretreated rats, SC challenge with AMP or OXZ produced stimulation indices of 10 and 20-fold, respectively, relative to SC injection of respective vehicle. Oral pretreatment with AMP (all doses) or OXZ decreased SC-induced proliferation approximately 63% and 81%, respectively, relative to vehicle pretreated rats. The percent of Tregs (CD4+FoxP3+) in the draining lymph node did not change substantially, but there was a slight increase in the 30 mg/kg AMP and OXZ groups. These data indicate that oral pretreatment with AMP can induce immune tolerance in rats. While the mechanism of oral tolerance is unclear at this time, this initial study suggests that it may be possible to develop a nonclinical model for the induction of oral tolerance that could be relevant to the study of drug allergic responses.

1930 SAFETY EVALUATION OF A ONE-ARMED RECOMBINANT HUMAN ANTIBODY TO C-MET (METMAB) IN CYNOMOLGUS MONKEYS.

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MetMAb is a novel recombinant humanized monovalent antibody that blocks hepatocyte growth factor (HGF) signaling through the receptor tyrosine kinase c-Met and is currently in Phase 1 trials as a therapeutic candidate for use in cancer patients. To enable Phase 1 clinical trials, cynomolgus monkeys were administered MetMAb weekly by intravenous injection for 13 doses. Toxicity was assessed by clinical observations, body and organ weight changes, ophthalmic examinations, hematology, coagulation profiles, serum chemistry, FACS analyses of B, T, and NK cells, urinalyses, macroscopic and microscopic pathology. Safety pharmacology evaluation included analyses of body temperature, heart and respiratory rates, blood pressures and electrocardiograms. Drug-related effects were limited to a possible dose-related, infusion-associated mydriasis in a subset of treated animals, which generally resolved within 24 hours. There was an anaphylactoid reaction in one mid-dose female following dose 8, which was attributed to a high anti-drug antibody (ADA) titer. This animal had the highest ADA titers compared to all other animals. This reaction was not observed with any other animal that tested positive for ADAs. No other findings were attributed to drug exposure. Overall, MetMAb has been shown to have a good preclinical safety profile with possible test article-related effects restricted to infusion-associated mydriasis and one anaphylactoid reaction. The highest nonseverely toxic dose (HNSTD) was determined to be the highest dose tested in the study.

1931 IDENTIFICATION AND CHARACTERIZATION OF NOVEL AHR LIGANDS ACROSS SPECIES.

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The aromatic hydrocarbon receptor (AhR) is a ligand-activated transcription factor involved in the species- and tissue-specific effects of exposure to pollutants such as polycyclic and halogenated aromatic hydrocarbons, including 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, dioxin). Recently, a wide variety of chemicals structurally dissimilar to established ligands have been reported to activate the AhR, raising questions on the structural specificity/diversity of AhR ligands. Accordingly, we used a variety of AhR-dependent bioassay systems to identify and characterize new AhR agonists and/or antagonists. Gel retardation assays (GRA) and AhR-dependent reporter gene cell bioassays were used to screen 147 compounds, mainly therapeutic chemicals. GRA analysis using rat hepatic cytosolic AhR identified 13 compounds that stimulated AhR:[32P]DRE complex formation at levels >10% of that induced by TCDD. Screening with recombinant H1L1.1c2 (mouse hepatoma), H4L1.1c4 (rat hepatoma), and GP121.1c8 (Guinea pig intestinal) cell lines stably transfected with an AhR-responsive luciferase reporter plasmid identified 22 chemicals showing agonistic activity in at least one species: 12 were active in all three species, 5 in two species, and 5 in one species. Interestingly, leflunomide induced significantly higher reporter gene activity than that maximally induced by TCDD. Further, 13 chemicals increased and 9 inhibited TCDD-induced luciferase expression in at least one cell line. Only fenbenzadole increased TCDD response in all cell lines, 4 compounds inhibited TCDD activity in all species, 5 had opposing effects in different cell lines, and 8 increased TCDD response in one species only. A variety of novel and structurally diverse AhR agonists/antagonists that differ quantitatively and qualitatively in a species-/cell-specific manner were identified. The results highlight the complexity associated with attempts to predict/extrapolate the AhR response of a given chemical/ligand observed in one biological system to that of another.

1932 ASSESSMENT OF PRILOCAINE, LIDOCAINE, *o*-TOLUIDINE AND 2, 6-DIMETHYLANILINE DNA ADDUCTS FORMATION IN F344 RATS.

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Prilocaine (prilo) and lidocaine (lido) are widely used topical analgesics. Prilo is metabolized to o-toluidine (*o*-T) and lido to 2,6-dimethylaniline (DMA). *o*-T and DMA are tumorigenic in rats, in urinary bladder (UB) and nasal mucosa (NM), respectively. This study assessed DNA adduct formation by prilo and lido in these tissues as well as liver and white blood cells in male F344 rats comparing with *o*-T and DMA all at equimolar doses. In addition, the putative genotoxic metabolites *N*-HO-*o*-T and *N*-HO-DMA were synthesized and reacted with DNA to provide reference DNA adducts. Adducts were measured by the ^{32}P-postlabeling assay. Groups

of 3 rats were treated either once or 7 times by oral gavage with 0.5 mmole/kg bw of prilo, lido, *o*-T or DMA or the vehicle (5 ml/kg bw of 0.5% carboxymethylcellulose, pH 6-7 with HCl) as control. All rats were sacrificed 24 hr after the final treatment. The cumulative 7 day doses of prilo and lido were about 40 to 50 times the human medicinal doses. No adduct was detected in any of the white blood cell samples. Liver and NM adducts were detected after administration of either prilo or lido for 7 daily doses. UB epithelium DNA adducts were not detected in the prilo-treated group. Groups treated with *o*-T or DMA showed adducts in both single and multiple dose groups. In the multi-dose group, the NM adducts of DMA were significantly higher than in the liver and also higher than with other test compounds in liver, NM and UB epithelium and ~10-fold higher than that of the DMA single dose group. The prilo and lido DNA adducts detected were indistinguishable from those formed in *o*-T- or DMA-treated rats or by chemical reaction of the corresponding *N*-HO derivatives with DNA. Thus, prilo or lido can lead to DNA adduct formation in liver and NM but only lido leads to adducts in the UB epithelium, although all at lower levels than equal molar quantities of the corresponding amines. That DNA adducts can be detected in rats treated with prilo and/or lido, albeit at doses much higher than those used clinically, warrants further investigation.

1933 LIVE CELL IN-VITRO SCREEN FOR DETECTING OXIDATIVE STRESS.

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Oxidative stress is caused by an imbalance between the production of reactive oxygen and nitrogen species (ROS and RNS respectively) and a biological system's ability to detoxify the reactive intermediates. Oxidative stress is involved in many diseases, such as diabetes, atherosclerosis, neurodegeneration as well as cardiovascular and liver injury. An important source of reactive oxygen in aerobic organisms is the leakage of activated oxygen from mitochondria during oxidative respiration. In an effort to reduce compound attrition due to liver toxicity, we have developed a multi-parametric assay to determine the potential of lead compounds to induce ROS using a dihydroethidium (DHE) probe in an immortalized human liver epithelial (THLE) cell line, following a time-dose response curve. Overall cell health was established by monitoring mitochondrial inner membrane potential ($\Delta\Psi m$), and cell number (using TMRM and Hoechst 33342 respectively). The assay was developed using compounds known to induce mitochondrial mediated ROS formation. Rotenone and doxorubicin induced ROS formation followed by dissipation of $\Delta\Psi m$ and cell death. FCCP induced ROS formation, loss of $\Delta\Psi m$ and cell death concurrently. Oligomycin which diminishes $\Delta\Psi m$ and induces cell death prior to ROS generation, was utilized to demonstrate a cause and effect relationship. The utility of the assay in the early drug discovery setting was demonstrated by evaluating 150 compounds known to induce liver or other organ toxicity and 40 non-toxic drugs at clinically relevant doses. None of the 40 non-toxic drugs induced ROS or cell death at clinically relevant doses. However, 30% of toxic compounds generated ROS prior to inducing cell death (suggesting a potential mechanism of toxicity); 4% depleted $\Delta\Psi m$ prior to ROS generation and cell death, and 16% generated ROS, depleted $\Delta\Psi m$ and cell death concurrently. In summary, the data show that ROS generation can be easily monitored and quantitated. The information can be utilized to help drive chemistry away from compounds that induce ROS generation, mitochondrial dysfunction or cell death.

1934 THE EVALUATION OF ATACICEPT ON PROTECTIVE IMMUNITY IN THE MOUSE STREPTOCOCCAL HOST RESISTANCE MODEL.

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Atacicept, a recombinant fusion protein that inhibits mature B cell survival and lowers serum immunoglobulin (Ig) levels, is being investigated clinically as a treatment for several autoimmune diseases and advanced B cell malignancies. Because of the role of serum Ig on bacterial clearance, a study was conducted to evaluate the effect of atacicept on protective immunity to bacterial infection.

C57BL/6N mice received SC injections of vehicle (negative control) or atacicept (0.1, 1, or 10 mg/kg) thrice-weekly from Days 1 through 36, or cyclophosphamide (CY, positive control, 200 mg/kg IP) on Days 19 and 22. Mice were infected intranasally on Day 23 (1 day post-dose) with 50 microliters (7-8 x 106 CFU) of Streptococcus pneumoniae, Type 14. Data were collected through Day 37 on mortality, lung bacterial clearance, peripheral blood lymphocyte subsets, serum total and streptococcal-specific IgG and IgM levels, and anatomic pathology.

As expected, vehicle-treated mice had bacterial counts that were maximal on the day of infection and cleared within two days. These mice mounted a streptococcus-specific Ig response and had good survival. CY treatment increased mortality, impaired bacterial clearance, and reduced total and streptococcus-specific Ig levels as compared to vehicle treated mice. Atacicept treatment decreased peripheral blood B cells, reduced splenic lymphocytes, and reduced serum total and streptococcus-specific Ig levels. However, bacterial clearance and overall survival were similar to vehicle-treated mice. Evaluation of the lungs from mice treated with vehicle and atacicept (10 mg/kg) demonstrated a similar mild pneumonia.

Under the conditions of this model, thrice-weekly SC injection of up to 10 mg/kg atacicept decreased B cells and total and streptococcus-specific IgG and IgM levels. Despite these effects, atacicept-treated mice survived and cleared streptococcal infection of the lung in a manner similar to vehicle-treated mice.

1935 TOXICITY ASSESSMENT WITH KINASE INHIBITORS.

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Protein kinases play a crucial role in signal transduction, in cellular proliferation and differentiation, and in various regulatory mechanisms. The inhibition of growth-related kinases, especially tyrosine kinases, might therefore provide new therapies for diseases such as cancer. Thus targeted kinase therapies are expected to show a high specificity toward tumor cells, providing a broader therapeutic window with less toxicity. Although the specific targeted mechanism of action of these new molecules is intended to maximize the therapeutic index, target organs not pharmacologically driven can emerge during preclinical safety studies. Indeed, as a consequence of treatment with such molecules, some protein-kinases that are involved in fundamental biological functions could also be inhibited in normal tissues, whereby a secondary pharmacological event can produce the onset and development of an adverse effect or a toxicological liability, either morphological or functional.

The use of a combination of different tools, from targeted histopathology endpoints, to electron and confocal microscopy and molecular profiling techniques performed during standard toxicology studies as well as in ad hoc designed studies, allows the determination of specific on- and off -target toxicities. For example, screening for gene and protein expression of different kinases in healthy tissues of different animal species in parallel with humans can give mechanistic understanding of induced toxicities, and in turn provide some predictive indications of the relevance of specific toxicities in patients.

Some examples with multiple kinase inhibitors (e.g. early ultrastructural impairment of the capillary microvasculature in target organs), and cyclin dependent kinases (e.g. increase in autophagic vacuoles in pancreatic acinar cells, peripheral nerve toxicity and retinal degeneration of photoreceptor layer) are presented.

1936 SUBCHRONIC ORAL TOXICITY OF THE CORTICOTROPIN-RELEASING HORMONE (CRH) RECEPTOR ANTAGONIST, ANTALARMIN, IN RHESUS MONKEYS.

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Antalarmin (AA; N-butyl-N-ethyl-[2,5,6-trimethyl-7-(2,4,6)-trimethylphenyl)-7H-pyrrolo[2,3-d]-pyrimidin-4-yl]amine) is a CRH type 1 receptor antagonist being developed as a treatment for stress-related pathologies. In a 14-day range-finding study in rhesus monkeys (1/sex/group), AA doses of 40, 80, or 160 mg/kg/day induced no gross clinical evidence of toxicity. In a follow-on repeat-dose pilot study, monkeys (2/sex/group) received AA at 300, 400, or 500 mg/kg/day. One monkey receiving AA at 500 mg/kg was euthanized *in extremis* on Day 1. Tremors and ataxia were observed in all dose groups; severity of CNS effects increased with dose and duration of exposure. In the definitive toxicity study, monkeys (3/sex/group) received AA doses of 0, 30, 100, or 300 mg/kg/day for 14 days. Endpoints included survival, clinical observations, body weight, body temperature, food consumption, electrocardiography, ophthalmology, clinical pathology (days 4, 8, 15), plasma drug levels (days 1, 8, 14), organ weights, and gross and microscopic pathology. AA had no effects on survival, body temperature, clinical chemistry, hematology, coagulation, organ weights, histopathology, or electrocardiographic or ophthalmologic endpoints in any group. Clinical observations were limited to tremors seen in the high dose group (300 mg/kg/day) only. Small but statistically significant reductions in body weight (females) and body weight gain (males) were seen in groups receiving AA at 100 or 300 mg/kg/day. Based on mortality seen at 500 mg/kg/day, the Maximum Tolerated Dose (MTD) for repeat-dose administration of AA to rhesus monkeys is 400 mg/kg/day (4800 mg/m^2/day). Based on body weight effects seen at doses $\geq$ 100 mg/kg/day, the No Observed Adverse Effect Level (NOAEL) for repeat-dose administration of AA to rhesus monkeys is 30 mg/kg/day (360 mg/m^2/day). (N01-CM-87102)

1937 STRATEGY FOR QUALIFYING HUMANIZED MICE AS TOXICITY MODELS IN METABOLITE SAFETY ASSESSMENT.

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Currently, studies utilizing direct dosing of metabolite must be considered for assessing the safety of drug metabolites formed uniquely or disproportionately in humans. Such studies introduce additional costs and delays as well as result in data with questionable utility. These factors may ultimately hinder getting needed drugs to patients. One alternative is to administer parent molecule to mice expressing humanized CYP450s. The overall goal of our current project is to determine the ability of genetically engineered mouse models that express human CYP450s to generate metabolite profiles more closely associated with humans than wild-type mice. The initial focus is developing an *in vitro* screen for qualifying these models. The specific aims associated with this focus are 1) identifying substrates with expected unique human metabolites or metabolites disproportionately formed in humans, 2) comparing metabolite profiles using liver microsomes from humans and wild-type mice, and 3) confirming *in vitro* results with *in vivo* studies.
The *in vitro* screen involves incubating liver microsomes with substrate for 30 minutes at 37°C. Protein precipitation is achieved through the addition of acetonitrile followed by centrifugation. A qualitative metabolite profile is generated by the application of high resolution accurate mass LC/MS utilizing narrow width (10mDa) extracted ion chromatograms (XIC). Preliminary results indicate that, of the initial substrate list, diclofenac and dexamethasone may have metabolites which are uniquely or disproportionately formed in humans when compared to wild-type mice. Further work is needed to verify and expand on these results.
In summary, we have initiated development of a high-throughput *in vitro* screen for qualifying humanized mouse models. While additional substrates must be identified and evaluated, we have taken an important step towards developing a strategy to qualify alternative models for use in assessing drug metabolite safety.

1938 THE USE OF OXYGEN AND PH SENSITIVE FLUORESCENT PROBES IN THE INVESTIGATION OF PERTURBED CELLULAR METABOLISM.

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In standard in vitro systems, the main sources of cellular ATP are Glycolysis and Oxidative Phosphorylation (OxPhos). The balance between these two related energy generating systems can therefore inform on disease states and toxicities where perturbed metabolism is implicated. A highly informative approach to the investigation of such metabolic perturbations is to measure both cellular oxygen consumption and extracellular acidification. Analysis of oxygen consumption gives specific information on OxPhos, while measurement of extracellular acidification, under the appropriate conditions, provides information on non-aerobic metabolism.
Here we present the use of a water-soluble, pH-sensitive fluorescent probe for the assessment of extracellular acidification of both adherent and suspension cells in a 96 well plate format. As probe emission lifetime is used as the readout, a simple transformation, allows the generation of extracellular acidification rates in units of [H+] per unit time. We also outline how these probes may be combined with the MitoXpress oxygen consumption assay providing a highly informative dual-parameter metabolic analysis. Such parallel measurements allow inferences to be drawn regarding the site of an observed metabolic insult; thereby allowing altered glycolytic activity to be delineated from direct mitochondrial effects. We examine how such an analytical approach may be deployed for the examination of the perturbed metabolism using compounds of known metabolic impact including antimycin, 2 deoxyglucose, oxamic acid and dichloroacetate as models. This analysis is then extended to more relevant models analyzing the effect of a panel of biguanides on cellular metabolism and relating these observations to their proposed mechanism of action.

1939 LACK OF EFFECT OF LACTATE DEHYDROGENSASE AND LACTIC ACID IN THE ENZYMATIC METHOD FOR ETHYL ALCOHOL DETERMINATION.

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The predominant method for detection of plasma ethyl alcohol (ETOH) in a hospital setting is via enzymatic assay. Previous studies from other laboratories have suggested that the presence of extremely high plasma levels of lactate dehydrogenase (LDH) and lactic acid may cause false positives in a number of enzyme ethanol detection systems. To assess the likelihood of false positives and/or elevated alcohol levels, a series of experiments were conducted in our laboratory using human plasma and various concentration of LDH and lactic acid. Samples of human plasma were spiked with LDH (Lee Biosolutions - rabbit muscle) and lactic acid (Sigma) and then measured on a Hitachi 717 with the Dade Behring Syva EMIT II Ethyl Alcohol assay. Data from repetitive in vitro measurements indicate that levels of LDH up to 10,000U/L in the presence of 10mM lactic acid showed no levels of ETOH above the assay's threshold (<10mg/dl). Samples of plasma spiked with 30mg/dl of ETOH, along with the added levels of LDH and lactate, showed no significant alteration in the detection of ETOH compared with controls. These data indicate that even with extremely high LDH and lactate levels, the Dade Behring Syva EMIT II Ethyl Alcohol assay is a reliable and accurate method to detect ETOH levels in plasma.

1940 NNC55-0396, A POTENTIALLY SAFE THERAPEUTIC T-TYPE CA2+ CHANNEL-SPECIFIC ANTAGONIST, DOES NOT STRONGLY INHIBIT CYP3A4.

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Mibefradil was clinically used as a potent T-type Ca2+ channel inhibitor for cardiac indications, but was withdrawn from the market after serious drug-drug interaction toxicity emerged, particularly due to inhibition of CYP3A4. Mibefradil also has the disadvantage of L-type Ca2+ channel antagonism, which emerges with its hydrolyzed metabolite, Ro 40-5966. Our results confirm that mibefradil is a potent inhibitor of CYP3A4 and CYP2D6, with IC50 values of 33 nM and 120 nM, respectively. We also found that the hydrolyzed metabolite, Ro 40-5966 is a potent inhibitor of CYP3A4 and CYP2D6 with IC50 values of 30 nM and 46 nM, respectively, which may have contributed to the overall toxicological complications of mibefradil. In contrast, NNC55-0396, a novel T-type Ca2+ channel inhibitor, has a similar structure to mibefradil, but its side chain is resistant to hydrolysis, so that it is a selective inhibitor of T-type Ca2+ channels. We found that NNC55-0396 is nine-fold less inhibitory toward CYP3A4 than mibefradil and Ro 40-5966 with an IC50 of 300 nM. NNC55-0396 inhibits CYP2D6 with approximately four-fold more potent than mibefradil, but equipotently to Ro 40-5966. Finally, all three compounds inhibit CYP2C9, CYP2C19, and CYP2C8 but only strongly at high concentrations (10 μM). Our results suggest that NNC55-0396 may be a potentially safer therapy than mibefradil as a T-type Ca2+ antagonist because, not only is it more selective, but clinically significant drug-drug interactions may not likely ensue when NNC55-0396 is co-administered with compounds which are cleared primarily by CYP3A4-mediated pathways.

1941 EVALUATION OF A NOVEL HIGH CONTENT BIOLOGY-BASED METHOD TO DETECT COMPOUNDS WITH STEATOTIC POTENTIAL.

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The present work describes the validation of an *in vitro* assay that detects hepatocellular steatosis using primary rat hepatocytes. This assay utilizes a high-content-biology-based cell analysis that detects palmitic acid labeled with the fluorescent dye bodipy (BPA) as an indicator of lipid retention. Prior to compound treatment, hepatocytes are cultured in the presence of oleic acid and BPA for 24 hours to achieve a baseline level of lipid inclusion within the cells. Lipid-laden cells are then treated with compound for 24 hours and then assayed for fluorescent lipid retention. Fluorescence fold-difference between treated and vehicle control is calculated and used as an indicator of steatotic potential. This model was validated using forty-eight compounds include 29 agents known to cause steatosis in humans by various mechanisms, 15 known hepatotoxicants that are not associated with steatosis, and 4 compounds that are neither steatotic nor hepatotoxic. Twenty-one of 29 steatotics (steatosis positive) tested positive, yielding a sensitivity of 72%. Excluding those compounds that inhibit DNA synthesis, none of which were positive, the sensitivity increases to 21 of 25 presumed positives, yielding a sensitivity of 85%. The specificity measure of this assay was calculated using compounds that are known hepatotoxicants and those that are not hepatotoxic. The results show 14 of 19 compounds called negative in this assay, yielding a specificity of 73%. However, when considering only hepatotoxic compounds the specificity is reduced to 62%. The ability of this assay to correctly detect compounds not known to cause steatosis (steatosis negative) in humans is confounded by cytotoxic effects *in vitro*. This model represents a novel method for detecting steatotic compounds using the retention of a fluorescent lipid in a pool of lipid within the cell and stringent exclusion criteria for cytotoxicity based on nuclear fluorescent intensity.

1942 CUTANEOUS CLINICAL ADVERSE DRUG REACTIONS IN A TERTIARY CARE CENTER.

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The present study was aimed at the clinical spectrum of cutaneous adverse drug reactions (ADRs) in hospitalized patients and to establish a causal link between the drug and the reaction by using WHO causality definitions. A prospective hospital-based study over a period of one year was carried out. The cutaneous ADRs of the patients admitted in the medical centre were recorded. The data were subjected descriptive analysisto .
RESULTS: A total of 55 patients diagnosed to have cutaneous ADRs were included in the study. Only drugs having certain and probable causal association with the reaction were considered for analysis. One reaction had certain causal association while 45 patients fell into the category of probable association. The most common types of ADRs were maculopapular rash (35%), followed by toxic epidermal necrolysis (TEN) (20%) and Stevens-Johnson syndrome (SJS) (15%). The drugs implicated for cutaneous ADRs were antiepileptics (44%), chemotherapeutic agents (32%), NSAIDs (11%). Antiepileptics were responsible for causing the maximum number of maculopapular rash (56%), TEN (55%) and SJS (43%). The reaction times for all these reactions were in accordance with the previous reports that confirm the causality of the suspected drug.
CONCLUSION: The occurrence of cutaneous ADRs in the present study was similar in many ways to studies conducted by various research workers earlier. A wide clinical spectrum of cutaneous ADRs ranging from mild maculopapular rash to serious SJS and TEN was observed. The incidence of life-threatening cutaneous ADRs like SJS and TEN was found to be higher compared to studies published abroad. Antiepileptics were implicated in the majority of the hospitalized cutaneous ADRs. Infrequently reported adverse reactions for newer drugs like leflunomide, cefotaxime and azithromycin were also detected in the present study.

1943 MECHANISTIC DIFFERENCES IN TOXICOLOGICAL RESPONSE TO PHOSPHOLIPOGENICS BY DOG AND RAT HEPATOCYTES.

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Toxicity responses of rat and dog hepatocytes to phospholipogenic drugs in vitro were investigated, and important species differences were revealed. Tested drugs included fluoxitine (F), perhexiline (PX), amiodarone (A), hydroxychloroquine (HCQ), and AstraZeneca (AZ) compounds, all known to cause hepatic phospholipidosis in vitro and in vivo. Hepatocytes were treated for 24 hr, and several endpoints were measured, including survival, ATP, JC-1 (a marker of mitochondrial membrane permeability), and accumulation of NBD-PE, a surrogate fluorescent marker of phospholipid accumulation. Dog cells were more sensitive to all treatments, as measured by indicators of cell survival, and were less able to mount phospholipogenic responses. Dog cell NBD-PE accumulation (extent) never exceeded 3-fold vehicle controls (VC), whereas rat cells accumulated NBD-PE up to 10-fold that of VC. In contrast, the potencies of NBD-PE accumulation induction (EC50's) were often greater in dog cells. Cotreatment with bafilomycin (Baf), a vacuolar ATPase inhibitor that is known to increase lysosomal alkalinity, rescued dog cells against less potent phospholipogenics (AZ's, HCQ, A) preferentially over potent ones (F, PX). Rat cells were not subject to rescue by Baf, even as NBD-PE maximal responses were concomitantly suppressed for all phospholipogenic compounds. Changes in JC-1 were consistent in rank order with potency- and species-specific findings for survival. In contrast, changes in ATP did not exhibit species specificity or correlation with phospholipogenic potency. Mitochondrial toxicity occurred within the same time frame as NBD-PE changes, suggesting that direct mitochondrial toxicity may also be invoked as a mechanism of toxicity. Taken together, the data suggest that hepatocytes with a lower capacity to mount a phospholipogenic response are more susceptible to cell death mediated by lysosomal destabilization and/or mitochondrial toxicity, whereas, paradoxically, the potency of phospholipogenic response appears to correlate with a greater sensitivity to toxic insult.

1944 ACETAMINOPHEN-PROTEIN ADDUCTS ARE NOT SUFFICIENT FOR HEPATOCELLULAR NECROSIS IN MICE: MODULATION OF TOXICITY BY GENDER AND GLUTAMATE CYSTEINE LIGASE MODIFIER SUBUNIT.

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Bioactivation of acetaminophen (APAP) results in hepatocellular necrosis under conditions of sustained glutathione (GSH) depletion. GSH synthesis is in-part limited by glutamate cysteine ligase (GCL), which is most active as a heterodimer of catalytic and modifer (GCLM) subunits. Disruption of one or both copies of Gclm leads to slightly impaired GCL activity, or to severely impaired GCL activity with depleted basal GSH, respectively, compared to wild-type (WT) mice. In this study, fasted male and female Gclm WT, heterozygous (HZ), and null C57BL/6 mice were treated with 300 mg/kg APAP. Liver damage (serum alanine aminotransferase activity), hepatic APAP-protein adducts (APAP-Cys by HPLC-electrochemical detection), and total GSH levels were assessed 6 hours post-APAP. All male mice exhibited marked and comparable liver damage, but an increase in APAP-Cys with decreasing Gclm. Female mice, however, demonstrated liver damage that was inversely associated with Gclm gene dosage, with little damage in female WT mice. Interestingly, APAP-Cys in female mice were variable, but comparable to that in male WT and HZ mice. In all mice, total GSH was slightly lower than controls. Further investigation in male HZ mice at 0.5, 1, and 2 hr following APAP treatment showed both rapid depletion of total GSH and formation of APAP-Cys. Liver damage was apparent at 6 hr with partial repletion of GSH yet the presence of APAP-Cys. Administration of N-acetyl-L-cysteine (NAC, 800 mg/kg) at 1 or 2 hr post-APAP decreased the levels of APAP-Cys and liver damage, and enhanced GSH repletion at 6 hr. NAC at 4 hr post-APAP partially protected. Regarding APAP toxicity, these results suggest: 1) the gender difference in C57BL6 mice is not predicted by 6-hr liver APAP-protein adducts; 2) GCL activity contributes to the resistance of female C57BL/6 mice; and 3) subsequent to APAP-protein adduct formation, liver damage may be mitigated by administration of NAC.

1945 CHRONIC ETHANOL ADMINISTRATION RESULTS IN DOWN REGULATION OF PEROXIREDOXIN 6 AND OXIDATIVE STRESS.

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Alcoholic liver disease is a multifactorial disease and oxidative stress is believed to play an intimate role in the initiation and progression of this pathology. Peroxiredoxin 6 (Prx6) is a cytosolic antioxidant protein that has the ability to scavenge hydrogen peroxide and organic hydroperoxides, such as phospholipid hydroperoxides. The goal of this study was to investigate the effect that chronic ethanol treatment has on inducing oxidative stress in the liver and how this treatment affects Prx6 expression in the mouse liver. After nine weeks of treatment with an ethanol containing diet, significant increases in serum ALT activity, liver to body weight ratio, liver triglycerides, CYP2E1 protein expression, CYP2E1 activity and 4-hydroxynonenal and 4-oxononenal protein adducts were observed in the ethanol treated animals compared to pair fed controls. Also, significant decreases in total glutathione were observed. In addition, novel findings of decreased Prx6 protein and mRNA were observed in the ethanol treated animals compared to the pair fed controls. Lastly, NF-κB activity was found to be significantly increased in the ethanol treated animals. Concurrent with the increase in NF-κB activity, decreases in both MEK1/2 and ERK1/2 phosphorylation were also observed in the ethanol treated animals compared to the pair fed controls. Together, these data demonstrate that chronic ethanol treatment results in oxidative stress implicating NF-κB activation as an integral mechanism in the negative regulation of Prx6 gene expression in the mouse liver.

1946 THE NATURAL TRITERPENOID OLEANOLIC ACID AND A SYNTHETIC DERIVATIVE CDDO-IM PROTECT THE LIVER FROM HEPATOTOXICANTS AND INCREASE ANTIOXIDANT GENES THROUGH ACTIVATION OF NRF2.

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Over a decade ago, our laboratory showed that pretreatment with the natural triterpenoid oleanolic acid protected mice from hepatotoxicants. Mice pretreated with oleanolic acid and then administered bromobenzene, acetaminophen, carbon tetrachloride, thioacetamide, furosemide, phalloidin, colchicine, cadmium chloride, or D-galactosamine/lipopolysaccharide had decreased hepatotoxicity. Here, we elucidate a mechanism for such hepatoprotection. Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor, which upon translocation into the nucleus, is capable of inducing a variety of cytoprotective genes. Treatment of mice with oleanolic acid (90 mg/kg, i.p.), once daily for three days, increased Nrf2 nuclear translocation as determined by Western blot and immunofluorescence. Furthermore, oleanolic acid increased mRNA and protein expression of the Nrf2 target genes NAD(P)H quinone oxidoreductase 1 (Nqo1), glutamate-cysteine ligase, catalytic subunit (Gclc), and heme oxygenase-1 (Ho-1). Oleanolic acid also increased Nqo1 enzyme activity. In Nrf2-null mice, oleanolic acid did not change mRNA, protein, or activity of Nrf2 target genes. The recently synthesized triter-

penoid CDDO-Im prompted us to investigate whether it is also hepatoprotective against chemical-induced liver injury. As hypothesized, pretreatment with CDDO-Im (1 mg/kg, i.p.), once daily for three days, protected the liver from bromobenzene, thioacetamide, and acetaminophen induced injury as determined by serum alanine transaminase (ALT) activity and histopathology. In addition, CDDO-Im induced the Nrf2 target genes Nqo1, Gclc, and Ho-1 in a dose- and time-dependent manner. However, this protection and mRNA induction was ablated in Nrf2-null mice. These studies demonstrate that oleanolic acid and CDDO-Im protect the liver from chemical-induced injury by activating the antioxidant transcription factor Nrf2. (Supported by NIH ES09649, ES09716, ES07079, and RR021940).

1947 GLUTATHIONE DEPLETION ACTIVATES SPECIFIC MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) IN MICE.

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Mitogen activated protein kinases (MAPK) are activators of diverse cellular responses. In recent years it has been postulated that some of these responses are modulated by cellular redox state, maintained mainly by glutathione (GSH). In mammals, GSH tissue levels are different among organs; however GSH metabolism and regulation occurs through intra and interorgan mechanisms. Also, it has been reported that exposure to xenobiotic or to chemical depletors of GSH is reflected in MAPK activation in vitro. Thus, we hypothesized that changes in GSH levels in an organism should induce organ-specific responses through MAPK activation. To test this hypothesis, mice were treated intraperitoneally with L-buthionine-S-R-sulfoximine (BSO) to inhibit GSH synthesis. The time-related GSH depletion in the liver and kidney correlated with $p38^{MAPK}$ phosphorylation and induction of *thioredoxin 1*(*Tx-1*) transcription. This positive regulation was associated with nuclear translocation of NF-κB and ATF-2 and c-Jun phosphorylation in the liver, but only c-Jun phosphorylation in the kidney. Increased levels of GSH were observed in the brain together with extracellular regulated kinase 2 (ERK2) activation, Nrf2 nuclear accumulation, and increases in transcription of *Nrf2, xCT, γ glutamylcisteine synthetase (γGCSr)* and *Tx-1*. Pretreatment with MAPK inhibitors SB203580 and U0126, or addition of the exogenous thiol N-acetylcysteine abrogated both $p38^{MAPK}$ and ERK2 activation as well as downstream effects on gene expression. No effect on *γGCSr* was observed. These results indicate that in mice, GSH depletion is associated with organ specific MAPK activation. Also, the phosphorylation of ERK2 in brain suggests a differential and "protective" response to thiol depletion.

1948 INCREASED SENSITIVITY OF GLUTATHIONE S-TRANSFERASE P-NULL MICE TO CYCLOPHOSPHAMIDE TOXICITY.

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Major off-target effects of the anticancer drug, cyclophosphamide, include hemorrhagic cystitis and cardiotoxicity. The high toxicity of cyclophosphamide is attenuated by administration of thiol-containing nucleophiles, such as N-acetyl cysteine or reduced glutathione (GSH), implicating electrophilic metabolites. Previous studies show cyclophosphamide metabolism generates acrolein, a highly reactive α,β-unsaturated aldehyde. We propose that the systemic toxicity of cyclophosphamide could be attributed to acrolein generation, thus enzymes that detoxify acrolein protect against cyclophosphamide toxicity. Thus, we examined cyclophosphamide toxicity in wild-type (WT) mice and mice lacking glutathione S-transferase (GST) P, which is the most efficient catalyst for the conjugation of acrolein with GSH. Tissues (heart, kidney, lung, small intestine, stomach, urinary bladder) of GSTP-null mice had significantly lower GST activity toward 1-chloro-2,4-dinitrobenzene and ethacrynic acid. Treatment with cyclophosphamide (200 mg/kg, i.p) led to a significant decrease in diastolic blood pressure, increased plasma total and LDL cholesterol, increased plasma AST level, and decreased plasma albumin and total protein in GSTP-null, but not WT mice. Loss of GSTP-gene did not affect the P450-mediated production of acrolein by cyclophosphamide in isolated hepatic microsomes. Compared with WT mice, the GSTP-null mice were more sensitive to acrolein-induced (2-5 mg/kg, ip) hypercholesterolemia, hypouria, and hypoalbuminuria. Cardiomyocytes isolated from GSTP-null mice were more sensitive to direct acrolein exposure than those from WT mice. Collectively, these data reinforce the view that acrolein participates in cyclophosphamide toxicity, which is prevented by GSTP-mediated conjugation. Because polymorphisms in the human GSTP gene code for protein variants that differ in catalytic efficiency with acrolein, it is likely that hGSTP1 polymorphisms influence the systemic toxicity of cyclophosphamide. This work supported by USPHS ES12062 and ES11860.

1949 MICE THAT DO NOT EXPRESS GLUTATHIONE REDUCTASE ARE MORE SUSCEPTIBLE THAN ARE WILD TYPE MICE TO INFECTION WITH GROUP B STREPTOCOCCUS.

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The highly conserved expression across species of glutathione reductase (Gsr) is usually attributed to a pivotal role for Gsr in glutathione-dependent antioxidant defense mechanisms through reduction of glutathione disulfide. However, Gr180 (formerly $Gr1^{a1Neu}$) mice express no Gsr, but are at most minimally more susceptible than are wild type C3H/HeN mice to oxidant stresses, such as from continuous exposure to >95% O2. The present studies were to test the hypothesis that Gr180 mice are more susceptible than are C3H/HeN mice to live bacterial infections. Gr180 and C3H/HeN mice (6 to 10 weeks of age) were given 0.1 ml, ip, of $3.9x10^{4-9}$ cfu/mL of group B streptococcus (GBS) strain COH1 (a virulent encapsulated serotype III clinical isolate), and observed for signs of morbidity. Mice that exhibited sepsis were euthanized before succumbing to infection, and bacterial loads were characterized in spleen homogenates. The logs of the LD50s in Gr180 and C3H/HeN mice were 7.92 and 7.09 (8.3 and 1.2 x 10^7 CFU), respectively, indicating that the Gr180 mice were almost 6 times more susceptible than are C3H/HeN mice to GBS infection. The greater susceptibilities of Gr180 mice than of C3H/HeN mice to infection were not due to growth defects in vivo, as the GBS strain COH1 grew equally well in plasma obtained from the two strains of mice. Our results and the earlier report of defective respiratory bursts in Gsr-deficient neutrophils isolated from three siblings from a consanguineous marriage [Loos Blood, 48:53, 1976] indicate critical functions for Gsr in bactericidal defense mechanisms. The surprising health of the Gr180 mice, despite the highly conserved expression of Gsr across species, suggests that the critical selection pressures on Gsr expression normally arise from challenges by bacteria or other infectious agents to which the animals are not exposed in vivaria.

1950 EXPRESSION AND FUNCTION OF ALDEHYDE DEHYDROGENASE 3B1.

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Aldehyde dehydrogenase (ALDH) enzymes catalyze the detoxification of numerous endogenous and exogenous aldehydes. Nineteen ALDHs exist in the human genome and mutations in ALDH genes are the molecular basis of several diseases. The ALDH3B1 locus has recently been linked to schizophrenia. We have recently shown for the first time, using lysates from ALDH3B1-transfected cells, that the ALDH3B1 protein is catalytically active with various aldehyde substrates and protects HEK293 cells against cytotoxicity induced by lipid peroxidation-derived aldehydes. The goal of this study was to biochemically characterize and determine the expression and subcellular localization of ALDH3B1. Recombinant ALDH3B1 was isolated to 80-85% purity by affinity and ion-exchange chromatography and found to be active with various aldehyde substrates. Subcellular fractionation of mouse organs revealed ALDH3B1 to be a cytosolic enzyme. Western blot analyses demonstrated high expression in liver, kidney and brain and moderate expression of ALDH3B1 protein in various organs including spleen, lung, ovary and testes. Quantitative RT-PCR revealed ALDH3B1 mRNA expression at high levels in various organs including liver, kidney, and brain with highest levels in tongue. In conclusion, ALDH3B1 is a catalytically active, cytosolic enzyme highly expressed in various organs and may represent an important cellular defense against oxidative stress processes. (Grant support: AA016875-01; EY11490).

1951 ALDH7A1 IS A NOVEL ALDH THAT PROTECTS AGAINST OXIDATIVE DAMAGE.

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The human aldehyde dehydrogenase (ALDH) superfamily contains 19 enzymes involved in the NAD(P)+-dependent oxidation of endogenous and exogenous aldehydes. ALDH7A1 was first identified as an osmotic stress-induced protein in plants. In humans, mutations in ALDH7A1 are responsible for Pyridoxine-Dependent Epilepsy (PDE). The aim of this study was to characterize the subcellular localization, tissue distribution, and biochemical properties of mammalian ALDH7A1. Through sequence analysis and RT-PCR we identified a second novel ALDH7A1 transcriptional variant, which was found to contain a mitochondrial

leader sequence. Both ALDH7A1 transcript variants were found to be expressed at different ratios in a tissue specific manner. Results from Western blot analyses and IHC revealed high expression of ALDH7A1 protein in mouse kidney, liver, brain, and tongue and low expression in the heart, stomach, and lung. ALDH7A1 protein was found in the cytosol, nucleus and mitochondria. Confocal microscopy of HUVEC cells indicated a primarily nuclear localization of ALDH7A1. Human ALDH7A1 protein, baculovirus expressed and affinity purified, was found to be catalytically active with various aldehyde substrates. Finally, ALDH7A1 protects against osmotic stress-induced apoptosis. In conclusion, ALDH7A1 is a novel ALDH found in the cytosol, nucleus and mitochondria, which along with its substrate specificities suggest potentially tissue- and cell-specific biological roles against oxidative damage. (Grant support: EY11490).

1952 ORAL ADMINISTRATION OF POLAPREZINC, AN ANTI-ULCER DRUG, PROTECTS MICE AGAINST ENDOTOXIN SHOCK.

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Polaprezinc (PZ), a chelate compound consisting of zinc and L-carnosine (Car), is an anti-ulcer drug developed in Japan and widely used in clinical field. In the present study, we investigated whether PZ suppresses mortality, pulmonary inflammation and plasma nitric oxide (NO) levels in endotoxin shock mice after peritoneal injection of LPS as well as whether PZ and its components, zinc and Car inhibit NO production in murine macrophage RAW264 cells treated with LPS. Animal studies were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The survival rate of mice decreased to 20% at 96 h after LPS (40 mg/kg) injection. Oral administrations of mice with PZ (100 mg/kg) 2 h and 1 h before LPS increased survival rates to 80% and 55%, respectively. Serum zinc level 1 h and 2 h after administration of PZ to mice increased 10- and 8-fold of normal level, respectively. The number of total cells in bronchoalveolar lavage fluid 24 h after LPS increased 10-fold compared with vehicle control. PZ administration 2 h before LPS reduced the cell number by 70%. Compatibly, PZ improved LPS-induced histological changes in the lung such as inflammatory cell infiltration and interstitial edema with alveolar hemorrhage. PZ also inhibited the increase in plasma NO levels after LPS injection. However, PZ did not enhance the induction of heat shock protein (HSP) 70 after LPS administration. Treatment of RAW264 cells with PZ or zinc (10-100 μM) at 2 h before LPS (1 μg/ml) addition suppressed NO production at 24 h after LPS in a dose-dependent manner. Car, however, failed to inhibit NO production. These results indicate that PZ, in particular its zinc subcomponent, inhibits LPS-induced endotoxin shock via anti-inflammatory activity containing the inhibition of macrophage activation, but not HSP induction.

1953 THE ROLE OF REACTIVE OXYGEN SPECIES(ROS)IN AIRWAY HYPER-RESPONSIVENESS:THE EFFECTS OF 2-CYANO-3, 12-DIOXOOLEANA-1, 9(11)-DIENE-28-OIC ACID(CDDO).

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Background: It is projected that by 2020 roughly 30 million Americans (9%). Oxidant air pollutants and activation of cellular NADPH oxidase may play a role in asthma through the generation of intracellular reactive oxygen species (ROS). ROS induce cellular injury and promote inflammation and hypersensitivity that lead to airway hyperresponsiveness (AHR). 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid-TFEA (CDDO), a drug that appears to upregulate anti-oxidant responses, may be a useful anti-inflammatory agent through the prostaglandin J/Nrf2/ARE pathway.
Hypothesis: In this study it is hypothesized that ROS plays a part in AHR and treatment with CDDO would attenuate this response.
Methods: Balb-C mice were sensitized with ovalbumin 14 days prior to OVA challenge. Mice were then treated daily with CDDO and challenged with 1% OVA for 6 days and subjected to whole body plethsmography on the 7th day. On day 8 mice were sacrificed for collection of bronchoalveolar lavage (BAL), serum, whole lung tissue, and spleen.
Results: CDDO suppresses AHR and airway eosinophilia in a dose dependent fashion. CDDO also suppresses Ova-specific IgE formation. Higher concentrations of CDDO failed to further reduce AHR and airway inflammation.
Conclusion: Suppression of AHR with the use of CDDO suggests that oxidative stress may play a role in AHR and asthma. Previous studies have found that CDDO upregulates the Nrf2/ARE pathway causing Nrf2 to migrate to the cell nucleus inducing anti-oxidant gene transcription. The dose-dependent effect of CDDO on down-regulation of the inflammatory response is also consistent with the Nrf2/ARE hypothesis. Normal cellular redox function may be disrupted with the use of higher doses of CDDO. This may be in part to CDDO increasing the levels of Nrf2 to the point where an over-exuberant anti-oxidant response interferes with normal cellular regulatory function.

1954 DISTINCT ROLES OF REACTIVE OXYGEN AND NITROGEN INTERMEDIATES IN ACUTE LUNG INJURY INDUCED BY OZONE AND BLEOMYCIN.

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Acute lung injury induced by toxicants such as ozone and bleomycin is associated with macrophage accumulation in the tissue and the release of reactive nitrogen (RNI) and oxygen intermediates (ROI) which are known to damage cellular macromolecules. In these studies we analyzed potential mechanisms mediating lung injury induced by ROI and RNI. The pulmonary collectin, surfactant protein D (SPD), possesses both pro- and anti-inflammatory activities which are controlled by two cysteine residues in the hydrophobic tail. SPD has been shown to be involved in acute lung injury and is a target for both RNI and ROI. Acute exposure of mice to ozone (0.9 ppm, 3 h) or bleomycin (3 U/kg, it) resulted in posttranslational modification of SP-D; either sulfenylation to form SOH-SPD or nitrosylation to form SNO-SPD. The effects of these modifications on the biological activity of SPD were analyzed. Bronchoalveolar lavage fluid (BALF), which contains SPD, was collected 48 h after ozone or 8 days after bleomycin exposure. An Amplex Red/HRP assay was used to examine the effects of BALF on the production of hydrogen peroxide by THP-1 monocytes during a respiratory burst. Treatment of THP-1 monocytes with BALF from both ozone and bleomycin treated mice resulted in a two-fold increase in respiratory burst activity. Pretreatment of BALF with arsenite (20 mM) to reduce sulfenic acids, significantly decreased hydrogen peroxide stimulating activity. In contrast, pretreatment with ascorbate (2 mM) which reduces S-nitrosylated cysteine residues was only effective in abrogating the activity of BALF from bleomycin treated mice. These data suggest that lung injury induced by bleomycin involves both RNI and ROI, while ozone induced injury is mainly mediated by oxidative damage. Furthermore, posttranslational modification of SP-D may be an important mechanism whereby ROI and RNI generated following exposure to pulmonary toxicants contribute to injury and inflammation. Supported by NIH ES004738, ES005022, ES007148, AR055073 and HL074115.

1955 DISRUPTION OF ANTIOXIDANT DEFENSE AND DNA REPAIR SYSTEMS DURING 2-ACETYLAMINOFLUORENE-INDUCED HEPATOCARCINOGENESIS IN RATS.

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2-Acetylaminofluorene (2-AAF) is a genotoxic hepatocarcinogen that leads to tumor formation without further interventions. It is widely believed that the covalent interaction of metabolic derivatives of 2-AAF with DNA is a critical step in the initiation of tumorigenesis. However, it has been suggested that while the presence of DNA adducts per se is a necessary prerequisite for tumor initiation, it is not sufficient for tumor formation, which results from a much broader alteration in cellular homeostasis. The present study examined the effects of chronic 2-AAF exposure on hepatic cellular antioxidant and DNA repair systems. Male Sprague-Dawley rats were fed 0.02% 2-AAF for 6, 12, 18, or 24 weeks. In the livers of rats exposed to 2-AAF, catalase and Mn-containing superoxide dismutase (SOD) activities did not change, but there was a progressive decrease in the activity and expression of glutathione peroxidase (GPX). The activity of Cu, Zn-SOD, after an initial increase at 6 and 12 weeks, steadily declined to reach its lowest value by 24 weeks of exposure. Similar changes were observed in the expression of genes responsible for base excision DNA repair. Additionally, the ratio between oxidized and reduced glutathione increased, indicating an enhancement of oxidative stress in the livers of 2-AAF-exposed rats. There was also an increase in DNA strand breaks, suggesting that the accumulation of DNA lesions was associated with diminished antioxidant protection and DNA repair systems. These results indicate that chronic exposure to 2-AAF leads to oxidative stress, an imbalance of antioxidant and DNA repair systems, and an accumulation of DNA lesions. Any or all of these events may contribute to tumor promotion, as evidenced by the formation of GSTP-positive foci in the rat livers after 24 weeks of 2-AAF treatment.

1956 670 NM PHOTOBIOMODULATION NORMALIZES ANTIOXIDANT ENZYME ACTIVITIES SUPPRESSED IN STREPTOZOTOCIN-INDUCED DIABETIC RAT.

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Hyperglycemia causes oxidative stress in the liver and kidney in patients with diabetes. In the United States, 16 million people are diabetic and the number is increasing at a rate of 5% per year. Photobiomodulation in the far-red to near-infrared range (630-1000 nm) has been shown to accelerate wound healing, improve recovery from ischemic injury in the heart and attenuate degeneration in the injured retina and optic nerve. The beneficial effects of light therapy result, in part, from intracellular signaling mechanisms triggered by the interaction of the light with the mitochondrial photoacceptor molecule, cytochrome *c* oxidase. The present studies test the hypothesis that 670 nm photobiomodulation attenuates oxidative stress in the liver and kidney in a rodent model of type I diabetes, the streptozotocin-treated rat. Male Wistar rats were made diabetic with streptozotocin (50 mg/kg, ip), then exposed to 670 nm photobiomodulation at a dose of 6 J/cm^2 once per day for 18 days (acute) and 15 weeks (chronic). Liver and kidneys were harvested, flash frozen, and then assayed for markers of oxidative and energy stress, including glutathione reductase, glutathione peroxidase, superoxide dismutase, catalase, glutathione-S-transferase, reduced glutathione, lipid peroxidation, and ATP. Antioxidant enzyme activities decreased and lipid peroxidation levels increased in the diabetic rat liver while cellular ATP levels were unchanged. Both antioxidant enzyme activities and lipid peroxidation were normalized to control levels by light therapy in the liver. The results of this study suggest that 670nm photobiomodulation may reduce membrane lipid peroxidation and stimulate antioxidant protective pathways in the diabetes-induced rat model. This non-invasive treatment may alleviate the oxidative stress-associated acute and chronic diabetic complications and in other diseases that produce oxidative stress.

1957 SUBCHRONIC TOXICITY OF INHALED TOLUENE IN RATS: OXIDATIVE STRESS MARKERS IN BRAIN.

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The effects of long-term exposure to volatile organic compounds (VOCs), which is of concern to the EPA, are poorly understood, primarily due to insufficient information about human exposure and unreliable animal models. A sub-chronic inhalation study with multiple endpoints was conducted to seek effects of the VOC toluene in rats. Adult male Long-Evans rats (N=240) were exposed to toluene vapor at mean ± SD concentrations of 0 ± 0, 10 ± 1.4, 97 ± 7, or 995 ± 43 ppm for 6h/d, 5 d/week for 13 weeks. 24 hours after the last exposure, 6 animals per group were sacrificed and brains were taken on ice. Cortex, hippocampus, and striatum were dissected, quick frozen on dry ice, and stored at -80oC until analysis of oxidative stress parameters. The brain regions were homogenized in 20 mM Tris buffer (pH 7.4) and centrifuged at 8000 g for 20 min. The supernatants were assayed for total aconitase activity, glutathione (GSH) peroxidase, GSSG reductase, GSH transferase, glutamylcysteine synthetase, superoxide dismutase, and NADPH quinone oxidoreductase-1 using kits from Oxis International Inc. The results showed that sub-chronic inhalation of toluene by rats affected several oxidative stress markers. Total aconitase activity (involved in maintaining iron homeostasis and an indicator of DNA damage) was inhibited in striatum, increased in hippocampus, and unchanged in cortex. The effect of toluene exposure on superoxide dismutase, GSSG reductase and glutamylcysteine synthetase was greater in striatum than in the other brain regions. These results indicate that subchronic toluene exposure may induce oxidative stress in the CNS, which could be a molecular basis for its neurotoxicity. (This abstract does not necessarily reflect USEPA policy).

1958 SUPPRESSION OF DFP-INDUCED OXIDATIVE INJURY AND DENDRITIC DAMAGE IN RAT BRAIN.

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Prophylactic agents acutely administered in response to anticholinesterases intoxication can prevent toxic symptoms, including fasciculations, seizures, convulsions and death. However, anticholinesterases also have long-term unknown pathophysiological effects making rational prophylaxis/treatment problematic. We have used organophosphate DFP (diisopropylphosphorofluoridate) as a model for nerve agents and pesticide organophosphate acetylcholinesterase inhibitor, in testing the hypothesis that seizure-induced reactive oxygen and nitrogen species (ROS/RNS) generation and alterations in ATP mediate neuronal injury. Rats treated with DFP (1.25 mg/kg, s.c.) developed onset of toxicity signs within 10-15 min, which progressed to maximal severity of seizures and fasciculation within 60 min. At this time point, biomarkers of cerebral ROS generation, F_2-isoprostanes (F_2-IsoPs) and F_4-neuroprostanes (F_4-NeuroPs) were significantly increased ($p<0.01$) to 142% and 225% of control, respectively. Severe seizures also induced significant ($p<0.001$) depletion in high energy phosphates followed by three fold elevation of citrulline (a marker of RNS) in rat cerebrum. Quantitative morphometric analysis of pyramidal neurons of the hippocampal CA1 region 1 h following DFP exposure revealed significant decreases ($p<0.01$) in dendritic lengths and spine density to 30% and 58% of control, respectively. When rats were pretreated 30 min before DFP exposure with the antioxidants alpha-phenyl-N-tert-butylnitrone (PBN, 200 mg/kg, i.p.) or Vitamine E (100 mg/kg, i.p./day for 3 days) a significant attenuation in DFP-induced increases in F_2-IsoPs, F_4-NeuroPs, citrulline and depletion of HEP was noted. Furthermore, attenuation of biomarkers of oxidative damage following antioxidant pretreatment was accompanied by rescue from dendritic degeneration of pyramidal neurons in the CA1 hippocampal area. These findings support a prominent role of antioxidants in promoting neuronal survival following anticholinesterase-induced seizures (Supported by NINDS NS057223).

1959 A BIOTIN-HYDROXYLAMINE LABELING METHOD FOR THE DETECTION OF OXIDIZED PROTEINS USING WESTERN BLOT ANALYSIS AND MASS SPECTROMETRY.

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Increased levels of oxidative modifications of proteins are implicated in the etiology and pathology of cardiovascular diseases, neurodegeneration, and aging. However, the detailed analysis of oxidative protein modifications remains challenging. We developed a new efficient method for the identification of oxidatively modified proteins in biological samples which is based on the use of an aldehyde-reactive probe, N'-aminooxymethylcarbonyl hydrazino-D-biotin (ARP), in combination with Western blot analysis and mass spectrometry. ARP forms an oxime derivative with the aldehyde/keto group found in oxidatively modified proteins thereby introducing a biotin moiety. The biotin tag is detected by avidin affinity staining on the Western blot. After matching the blot images with the gels ARP-positive gel regions are excised and subjected to in-gel trypsin digestion. Protein identification is achieved by tandem mass spectrometry. In addition, biotin-modified oxylipid-peptide conjugates are amenable to enrichment using avidin affinity chromatography. Tandem mass spectrometry of the labeled oxylipid-peptide conjugates indicates that the biotin moiety is at least partially retained on the fragment ion during the collisionally induced dissociation experiments, a prerequisite for the use of automated database searching of uninterpreted tandem mass spectra. In the present study, we demonstrated the usefulness of the method for the analysis of carbonyl-modified proteins in heart mitochondria.

1960 A UPLC/MS/MS PROCEDURE FOR THE DETERMINATION OF ISOPROSTANES IN HUMAN SERUM.

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Reactive oxygen species can damage nucleic acids, proteins, and lipids. One of the most reliable indicators of lipid peroxidation in vivo are the isoprostanes, which are prostaglandins produced via the free radical-induced peroxidation of arachidonic acid. However, methods for measuring isoprostanes in human serum suffer from poor reproducibility and difficult preparation procedures. The aim of this project was to develop a method to measure 8-isoprostaglandin F2α (8-iso-PGF2α) in human serum using liquid chromatography-tandem mass spectrometry (LC/MS/MS). To accomplish this human serum was spiked with 8-iso-PGF2α and its deuterated internal standard and extracted using solid phase extraction. The sample was then injected into a Waters UPLC system equipped with an Acquity BEH C18 column. A gradient of acetonitrile and ddH2O was used for the chromatography. 8-iso-PGF2α and 8-iso-PGF2α-d4 were detected using a Waters Micromass Quattro Micro MS/MS operated in the negative ion, multiple reaction mode with the following transitions: 8-iso-PGF2α, m/z 353.20>193.25; 8-iso-PGF2α-d4, m/z 357.20>197.25. Using the above conditions we successfully separated the 8-iso-15(R) PGF2α (Retention time (Rt)=8.25 min), 15(R)-PGF2α (Rt=8.9 min), PGF2α (Rt=9.08 min), and 11β-PGF2α (Rt=9.08 min) isomers from our analyte of interest (8-iso-PGF2α; Rt=8.45 min). Our assay was linear from 25 pg/ml–10 ng/ml and the extraction efficiency ranged from 85-90%. Precision studies showed an intra-assay coefficient of variation (CV) of 8.7% and

an inter-assay CV of 11.3% at 50 pg/ml. The lower limit of detection was 10 pg/ml. Compared to a similar HPLC/MS/MS method (using a Waters XTerra MS C18 column) our assay offers an almost 50% reduction in the time required to elute 8-iso-PGF2α. In summary, we present an assay to quantify 8-iso-PGF2α in human serum that improves on previous work by offering high sensitivity and reproducibility in a short amount of time. Future studies will aim to establish a reference range for 8-iso-PGF2α in a normal population.

1961 PHOSPHOLIPID PEROXIDATION BIOMARKERS OF ALZHEIMER'S DISEASE IN THE BRAIN: SELECTIVE OXIDATION OF PHOSPHATIDYLSERINE.

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Oxidative stress and amyloid precursor protein (APP) are believed to significantly contribute to Alzheimer's disease (AD) pathology and plaque formation. While source(s) of oxidative stress have not been clearly identified, myeloperoxidase (MPO) is one of likely candidates. We have recently developed an oxidative lipidomics approach allowing identification and characterization of peroxidized phospholipids. We employed a combination of ESI-MS and fluorescence HPLC techniques to assess the amount of specific phospholipid hydroperoxides in post-mortem brain samples from AD patients and huMPO/APP transgenic mice (18 mo age). The pattern of phospholipid oxidation displayed a non-random character. Selective oxidation of phosphatidylserine (PS) was observed while other more abundant phospholipids -phosphatidylethanolamine and phosphatidylcholine – remained non-oxidized. ESI-MS analysis and tandem MS/MS experiments revealed that PS with oxidized C22:6 (m/z 866 (C18:0/C22:6+OO) originating from the ion at m/z 834 (C18:0/C22:6)) was the major oxidized molecular species in both AD patients and transgenic mice. Additionally, other oxidized molecular products of PS with m/z 850, 882, and 898 formed from C22:6 fatty acid and corresponding to molecular clusters (C18:0/C22:6+O), (C18:0/C22:6+O+OO) and (C18:0/C22:6+2OO), respectively, were detected. The content of PS hydroperoxides in brain samples from AD patients was 5-fold higher than in controls. In animals, the level of PS-OOH decreased in the order MPO+/App+>MPO-/App+>MPO+/App->WT>MPO knockout. This first demonstration of similarity between specific PS oxidation products in human and animal model of AD based on MPO-APP transgenic mice provide important insights into the mechanisms by which MPO participates in the promotion of AD pathology. Support: PA Dept of Health SAP4100027294.

1962 EVIDENCE OF OXIDATIVE STRESS IN LUNG, LIVER AND HEART FROM SHEEP EXPOSED TO CHLORINE (CL_2) GAS.

M. A. Dubick, A. I. Batchinsky, J. L. Barr and L. C. Cancio. *U.S. Army Institute of Surgical Research, San Antonio, TX.*

Toxic industrial chemicals such as Cl_2, are potential terrorist weapons, acting as a strong respiratory irritant that can lead to acute lung injury (ALI) defined clinically as a PO_2 to FiO_2 ratio < 300. As the pathophysiology of Cl_2 inhalation is incompletely understood, the present study investigated indices of antioxidant status in lung, liver and heart from anesthetized sheep (n=5-7/group) exposed to 0, 60 or 90 ppm Cl_2 in room air via a ventilator for 30 min. Sheep were euthanized at 24 hr after exposure and tissues were frozen in liquid N_2 until analyzed. Cl_2 exposure at both doses resulted in PO_2 to FiO_2 ratios indicative of ALI which persisted throughout the study period in sheep exposed to 90 ppm Cl_2. Despite the presence of ALI, thiobarbituric acid reactive substances (TBARS) in lung were only 16% higher than controls in sheep exposed to 90 ppm Cl_2. However, dose-dependent increases in lung glutathione peroxidase and copper-zinc superoxide dismutase (SOD) activities were observed following Cl_2 exposure compared to controls and reduced glutathione (GSH) levels were 26% and 47% higher than controls after exposure to 60 and 90 ppm Cl_2, respectively. Myeloperoxidase activity, as an index of neutrophil infiltration into lung, showed a similar dose-dependent increase after Cl_2 exposure. In contrast, lung catalase activity was 33% lower than controls after exposure to 90 ppm Cl_2. In liver, TBARS and manganese SOD activity were significantly higher, whereas GSH levels were over 20% lower than controls primarily after exposure to 90 ppm Cl_2. GSH levels in heart were also about 32% lower than controls after exposure to 90 ppm Cl_2. Taken together these data indicate that Cl_2 exposures as low as 60 ppm induce ALI and affect the antioxidant balance in lung 24 hr after exposure, while doses of 90 ppm produced a persistent ALI over the study period and may also induce a systemic effect.

1963 NRF2 PLAYS A PROTECTIVE ROLE IN RESPONSE TO PRO-OXIDANT EXPOSURE OF ZEBRAFISH EMBRYOS (*DANIO RERIO*).

L. Van Tiem, A. Timme-Laragy and R. Di Giulio. *ITEHP, Duke University, Durham, NC.*

Numerous toxicants generate reactive oxygen species (ROS) and cause oxidative stress, which can be particularly harmful to developing embryos. The antioxidant response element transcription factor NF-E2 p45-related factor (NRF2) plays an important role in responding to ROS by upregulating numerous antioxidant and Phase II metabolism genes. We hypothesized that NRF2 plays a protective role against oxidative stress in zebrafish. To examine this, we used a morpholino against NRF2 to knockdown its expression in developing embryos exposed to the model pro-oxidant tert-butyl hydroperoxide (tBOOH). Non-injected (NI), control-morpholino injected (Co-MO), and NRF2-morpholino injected (NRF2-MO) embryos were dosed with 0.1 - 2 mM tBOOH at 48 and 72 hpf. Hatching and survival were measured at 72 and 96 hpf. At both timepoints the LC_{50} for NI and Co-MO embryos was 1 mM while for NRF2-MO it was 0.75 mM at 72 hpf and 0.5 mM at 96 hpf. Hatching was also significantly reduced in NRF2-MO at both doses. In addition, NRF2-MO, Co-MO and NI embryos were dosed with 0 or 500 μM tBOOH at 24 and 48 hpf. Quantitative real-time PCR was used to measure the activity of redox-responsive genes MnSOD, CuZnSOD, pi class of glutathione-s-transferases (GSTp), glutathione peroxidase 1 (GPx1), and the catalytic subunit of glutamate-cysteine ligase (GCLc). In NI and Co-MO embryos, MnSOD induction was increased approximately 1.7-fold, GLCc 2.7-fold, GPx1 4-fold, and GSTp 7-fold. CuZnSOD was not upregulated in any group. tBOOH exposed NRF2-MO embryos were not able to induce any of the genes examined. These findings suggest that NRF2 serves a protective role in response to oxidative stress in zebrafish, and that as early as 48 hpf, embryos are capable of mounting an antioxidant response at the transcriptional level. This work also highlights the zebrafish as a model organism to study oxidative stress during embryonic development *in vivo*. This research was supported by the NIEHS-supported Duke University Superfund Basic Research Center (P42 ES10356), Integrated Toxicology Program (T32 ES07031) and EPA STAR.

1964 IS OXIDATIVE STRESS A SIGNIFICANT FACTOR IN THE SYNERGISTIC DEVELOPMENTAL TOXICITY OF MODEL PAHS IN ZEBRAFISH?

R. Di Giulio, A. R. Timme-Laragy, L. Van Tiem and D. Jung. *Environment, Duke University, Durham, NC.*

Early life stages of fish are sensitive to the toxic effects of polycyclic aromatic hydrocarbons (PAHs), which can cause pericardial edema and craniofacial malformations. We previously reported that certain combinations of PAHs result in synergistic rather than additive developmental toxicity, as in the case of co-exposure to the aryl hydrocarbon receptor (AHR) agonist β-naphthoflavone (BNF) and cytochrome P4501A (CYP1A) inhibitor α-naphthoflavone (ANF). In the current study, we hypothesized that oxidative stress contributes to this toxicity. We examined mRNA induction of several redox responsive genes in zebrafish embryos exposed to a range of concentrations of BNF alone and to ANF alone, to a BNF+ANF combination, and to a pro-oxidant positive control. We also measured total glutathione, nuclear and mitochondrial DNA damage, and also attempted to modulate deformities with pre-treatment to the glutathione synthesis inhibitor buthionine sulfoxamine (BSO) and antioxidant N-acetyl cysteine (NAC). Evidence of redox perturbation in this toxicity is manifested by significant increases in mRNA expression of MnSOD, CuZnSOD, glutathione peroxidase 1, the pi class of GST, and glutathione cysteine-ligase in BNF+ANF co-exposures at 55 hpf, but not in single doses of BNF or ANF used in the co-exposure. These induction levels were either higher or similar to induction levels from exposure to tert-butyl hydroperoxide (tBOOH). DNA damage measurements showed a trend towards more nuclear but not mitochondrial DNA damage in BNF, ANF, and co-exposure treatments. Decreased glutathione levels by BSO did not worsen deformities, nor was NAC able to rescue toxicity. While redox sensitive pathways were activated, additional studies are necessary to examine the role of oxidative stress synergistic developmental toxicity of PAHs. This research was supported by the NIEHS Duke University Superfund Basic Research Center (P42 ES10356), Integrated Toxicology Program (T32 ES07031), and EPA STAR to A.T-L.

S 1965 ARSENIC AND CARDIOVASCULAR DISEASE.

J. States[1] and A. Barchowsky[2]. [1]*Pharmacology & Toxicology, University of Louisville, Louisville, KY and* [2]*Environmental & Occupational Health, University of Pittsburgh, Pittsburgh, PA.*

Epidemiological studies indicate that chronic arsenic ingestion causes cardiovascular disease in humans. However, the mechanisms of arsenic-induced vascular diseases are unknown. Widespread exposure to arsenic in drinking water in the U.S.

likely contributes to atherogenesis and death from cardiovascular disease. This symposium will address mechanistic studies of arsenic-induced vascular disease in experimental mouse models and the influence of dietary factors in exposed humans. The presentations will focus on atherosclerotic changes in arsenic exposed mice, arsenic induced activation of signaling cascades triggering vascular remodeling, prenatal arsenic exposure induced epigenetic changes and epidemiological studies of dietary modification of arsenic induced cardiovascular disease.

S 1966 THE RELATIONSHIPS OF ARSENIC EXPOSURE FROM DRINKING WATER WITH MOLECULAR, PRECLINICAL, AND CLINICAL ENDPOINTS OF CARDIOVASCULAR DISEASE IN BANGLADESH.

Y. Chen[1], J. H. Graziano[2], F. Parvez[2], R. M. Santella[2], P. Factor-Litvak[2], V. Slavkovich[2], M. G. Kibriya[3] and H. Ahsan[3]. [1]*Environmental Medicine, New York University School of Medicine, New York, NY,* [2]*Environmental Health Science, Columbia University, New York, NY and* [3]*Health Studies, The University of Chicago, Chicago, IL.* Sponsor: A. Barchowsky.

Epidemiologic studies of cardiovascular disease (CVD) risk factors and appropriate biomarkers in populations exposed to a wide range of arsenic (As) levels are of a public health research priority. The Health Effects of Arsenic Longitudinal Study, a prospective cohort study with 11,746 participants in Araihazar, Bangladesh, was established in 2000 to evaluate health effects of As exposure from drinking water. IRB protocol approval has been obtained. We conducted cross-sectional, longitudinal, and cohort analyses to evaluate the associations of As exposure with blood pressure, proteinuria, plasma levels of soluble intercellular adhesion molecule-1 (sICAM-1) and soluble vascular adhesion molecule-1 (sVCAM-1), and CVD incidence and mortality. Arsenic exposure was positively associated with pulse pressure, especially among individuals with low intake of B vitamins and folate. There was a dose-response relationship between As exposure and proteinuria at baseline and between changes in As exposure over 2 years and incidence of proteinuria. In a randomized clinical trial of vitamin E and selenium supplementation with 115 HEALS participants with As-related skin lesions, baseline well As, a long-term measure of As exposure in the study, was positively associated with baseline levels of both sICAM-1 and sVCAM-1 and changes in the two markers over 6 months. Findings from a feasibility study of carotid artery intimal-medial thickness suggest a possible association. Preliminary prospective analysis suggests a dose-response relationship between As exposure and CVD fatal and non-fatal events. However, more follow-up is needed for definitive analysis. These findings suggest dose-dependent effect of As exposure on molecular, preclinical and clinical endpoints of vascular diseases and indicate potential mechanisms.

S 1967 ARSENIC-INDUCED ATHEROGENESIS: CONTRIBUTION OF OXIDATIVE STRESS, INFLAMMATION AND UNFOLDED PROTEIN RESPONSE.

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Chronic arsenic (As) exposure causes inflammation and peripheral vascular disease in humans. We examined the effect of early life and adult exposure to As on atherogenesis in apoE-/-mice maintained. For early life exposure, 3-week old mice were provided either tap water or water containing 1, 4.9 or 49 ppm As for 13 weeks. Plasma cholesterol of As-exposed mice was unchanged from controls, but plasma triglycerides were 10-25% lower. As-exposed mice had a concentration-dependent increase (1.5-3.0 fold) in lesion area in aortic sinus and arch. Similar to early life exposure, 10 week old adult mice provided water with 49 ppm As for 26 weeks had >2-fold increased lesion area throughout the aortic tree. Immunohistochemical analysis showed a 2-fold increase in macrophage accumulation in aortic valve and significant increases in staining for markers of inflammation (MCP-1), oxidative stress [protein-4,hydroxynonenal (HNE), protein-malondialdehyde (MDA)] and endoplasmic reticulum (ER)-stress and unfolded protein response (UPR;ATF3 and KDEL)in As-exposed mice. Plasma levels of HNE, MDA and pro-inflammatory cytokines (TNF-α, IL-1β, MCP-1, VEGF) were also significantly increased in As-exposed mice. In vitro, incubation of human umbilical vein endothelial cells (HUVEC) with 5-25 μM NaAsO2, resulted in time- and concentration-dependent increases in markers of oxidative stress, triggered UPR and induced the expression of pro-apoptotic protein, CHOP. Chemical chaperone, phenyl butyric acid completely abolished induction of ATF3. Collectively, our data suggest that As induces oxidative stress and ER-stress in endothelial cells and accelerates atherosclerosis and inflammation in apoE-/- mice. Supported by PHS grants R01HL65618, R01ES011314, PO1ES11860 and P30ES014443, & UofL Center for Genetics and Molecular Medicine pilot grant.

S 1968 PRENATAL ARSENIC EXPOSURE ALTERS HEPATIC DEVELOPMENTAL PROGRAMMING PREDISPOSING TO ATHEROSCLEROSIS.

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Cardiovascular disease rises in areas with elevated arsenic in drinking water. However, the extent to which developmental arsenic exposure plays a role in chronic adult diseases is uncertain. Prenatal arsenic exposure accelerates atherosclerosis in an ApoE-knockout mouse model. After intrauterine exposure to arsenic (85 mg/L $NaAsO_2$) via maternal drinking water on GD8 - GD20, offspring reared on normal chow have >2-fold lesion area in aortic roots and arch compared to control mice at 10 and 16 weeks of age. Total plasma cholesterol and phospholipids remained unchanged, but triglycerides were 20 - 40% lower in arsenic exposed mice. The role of altered developmental (fetal) programming in acceleration of atherogenesis was investigated by microarray-based analyses of mRNAs and microRNAs in livers on post-natal day (PND) 1 and PND70. Principal component analyses indicated that prenatal arsenic exposure altered the developmental trajectory of gene expression both for mRNAs and microRNAs The intersection of mRNAs with altered expression and targets of miRNAs with altered expression were subjected to DAVID analysis to identify altered pathways. PND1 mice showed dysregulation of gene expression in fundamental metabolic pathways including glycolysis and gluconeogenesis. Pathways for complement and coagulation cascades and for antigen processing and presentation were dysregulated in PND70 mice. These results are consistent with altered liver development and hepatic inflammatory responses that may contribute to the acceleration of atherogenesis caused by prenatal arsenic exposure in ApoE-knockout mice without a hyperlipidemic diet. (Supported by PHS grants R01HL65618, R01ES011314, P30ES014443, pilot grant from UofL Center for Genetics and Molecular Medicine, UofL Collaborative Planning and Development Grant and Intramural Research Program of NIA/NIH)

S 1969 SIGNALING MECHANISMS FOR VASCULAR RESPONSES TO ARSENIC.

A. Barchowsky[1], L. R. Klei[1], A. C. Straub[1] and D. B. Stolz[2]. [1]*Environmental and Occupational Health, Universtity of Pittsburgh, Pittsburgh, PA and* [2]*Cell Biology, University of Pittsburgh, Pittsburgh, PA.*

Arsenic has multiple poorly defined modes of action for promoting vascular diseases and tumor growth. Interactions of low levels of trivalent arsenic with protein thiols play a well recognized role in activating cell signaling and altering cellular metabolism. However, the signaling proteins that trigger and regulate the cellular actions of arsenic remain elusive. Studies using in vivo mouse models and primary endothelial cell cultures demonstrate that arsenic, even at exposures below the current drinking water MCL, selectively activates specific proximal signaling enzymes that regulate transcriptional activity. Stimulation of proximal GTPase and NADPH oxidase activities are required for oxidant-mediated transcriptional induction of cytokines and growth factor expression in human microvascular cells. In contrast, induction of adaptive responses, such as heme oxygenase-1 expression, is not regulated by arsenic-stimulated GTPase activity. Differences in gene expression reflect parallel activation of separate transcriptional programs and are only partially oxidant-dependent. The data support the hypothesis that arsenic has pleiotrophic actions that promote vascular remodeling and disease by inappropriately activating proximal proteins and enzymes in signal amplification cascades. Supported by NIEHS ES013781 (AB), EPA STAR FP-91654201 (AS) and NCI CA76541 (DS).

S 1970 NANOMATERIAL PHARMACOKINETICS AND PHARMACOKINETIC MODELING: WHERE WE ARE AND WHERE WE NEED TO GO.

N. Walker. *National Institute of Environmental Health Sciences, Research Triangle Park, NC.*

The rapid development and commercialization of new nanomaterial products including catalysts, cosmetics, drug delivery systems, presents an urgent need for toxicological assessment of these new submicron particulate products. Understanding

the unique kinetics of particle uptake, distribution and elimination in vivo and particokinetics in vitro are central to the safety assessment of nanomaterials. This symposium will present the latest findings related to the physiological and biochemical processes controlling nanomaterial pharmacokinetics as well as specific data on the portal of entry (skin, lung) and systemic kinetics of several important classes of nanomaterials including PEBBLEs, polystyrene spheres and quantum dots. The session will conclude by bringing together current empirical knowledge on the kinetics of nanomaterials with the material property and physiological determinants of tissue uptake to develop a framework for developing physiologically motivated kinetic models for nanomaterials.

S 1971 PHYSICAL AND BIOLOGICAL BARRIERS TO THE ABSORPTION OF NANO-SCALE MATERIALS.

P. J. Sinko. *Pharmaceutics, EM School of Pharmacy, Rutgers University, Piscataway, NJ.* Sponsor: J. Teeguarden.

Xenobiotic transport and uptake mechanisms across the intestinal, pulmonary and nasal mucosae and across the blood-brain barrier are reasonably well understood as they have undergone intense scrutiny in the pharmaceutical (i.e., drug delivery) and nutritional sciences over the years. However, with the unexpected realization that nano-scale materials (i.e., 1-100nm) can be rapidly absorbed and distributed widely throughout the body, these mechanisms require re-evaluation. This presentation will provide an in depth analysis of the barriers to and mechanisms of absorption and disposition of nano-scale materials and provide a rational framework for analysis and interpretation of absorption/exposure phenomena. Several case studies will be discussed including the ready absorption of nanoscale biologics (e.g., viruses), absorption/nonabsorption of materials and the relationship to potential absorption mechanisms. Also discussed will be the surprising absorption of non-nucleoside reverse transcriptase inhibitors that would be expected to be nonabsorbable based on their physicochemical properties (i.e., lipophilicity, charge and size). Finally, an analysis of the factors that control nanoscale materials body disposition and, ultimately, systemic and cellular exposure will be presented.

S 1972 SYSTEMIC KINETICS AND COMPARTMENTAL MODELING OF NANOMATERIALS.

M. Philbert. *Toxicology, University of Michigan, Ann Arbor, MI.*

PEBBLEs are nano-optical sensors or medical imaging devices with mean diameters in the range 60 – 150 nm. Sensors and image contrast agents may be made from hydrogels, liquid polymers or organically modified silicates and incorporate a variety of optically or magnetically active components. These are designed for systemic or intracellular physiological measurements. We have studied the kinetics of blood clearance of surface modified PEBBLEs and described their systemic kinetics using classical compartmental models. This talk will examine the utility of surface coating and biodegradability in avoiding the reticulo-endothelial system and altering the kinetics of elimination. These considerations may inform the vulnerability of internal compartments to accumulation and the increased potential for harm arising from exposure to engineered nanomaterials.

S 1973 BIODISTRIBUTION OF NANO AND MICRON SIZED FLUORESCENT POLYSTYRENE LATEX SPHERES FOLLOWING PARENTERAL OR LUNG EXPOSURE.

K. Sarlo. *Miami Valley Laboratories, Procter & Gamble Company, Cincinnati, OH.*

Limited information from work done with engineered nanoparticles suggests that these materials may move from a portal of entry (e.g., lung) and distribute to other tissues. Understanding tissue distribution and clearance of nano-sized particles following different routes of exposure is important to developing appropriate safety assessments. Female F344 rats received a single I.V. tail vein injection or single airway exposure via pharyngeal aspiration to 20nm, 40nm, 100nm or 1000nm fluorescent latex spheres and subsequent tissue distribution was assessed over 90 days. The fluorescent signal half-life was shorter for the 20nm and 40nm spheres compared to the 100nm and 1000nm spheres via both routes of exposure. Greater than 95% of signal was found in the liver and spleen for all 4 sizes following IV exposure. There was a significant shift in signal to the spleen over time for the 40nm and larger spheres but not for the 20nm spheres. The 20nm spheres were found in the circulation up to 7 days post-exposure while the 100nm and 1000nm spheres were found in bone marrow up to 90 days post-exposure. No signal was found in brain after 7 days post-exposure for any size sphere. More than 95% of signal was found in lung for all 4 spheres following airway exposure. While greater than 80% of the extrapulmonary signal was found in the gut 24 hours after exposure to 40nm and larger spheres, 50% to 60% of signal was found in blood for 20nm spheres. In contrast to 100nm and 1000nm spheres, there was little movement of 20nm and 40nm spheres to liver and spleen over time. Spheres 100nm and smaller were found in kidney. Spheres 40nm and larger were found in the uterus/ovaries with 40nm spheres detected at 2 months post-exposure. These data suggest that a single (acute) exposure to different size nanospheres leads to comparable tissue distribution with some subtle differences observed in extrapulmonary tissues. It is expected that repeat exposure studies can be used to understand the importance of these differences in tissue distribution.

S 1974 PHARMACOKINETICS AND PHARMACOKINETIC MODELING OF QUANTUM DOTS IN SKIN.

J. Riviere. *Veterinary Medicine, North Carolina State University, Raleigh, NC.*

This presentation will focus on quantitating the interaction between quantum dot (QD) nanoparticles and the skin, both from the perspective of penetration after topical exposure as well as distribution after systemic delivery. Biodistribution of QD in a perfused skin model showed particle coating dependency as well as a unique periodicity in arterial-venous extractions. A pharmacokinetic model describing QD skin distribution will be presented in great detail, including how a periodic function was incorporated into the model to accurately describe QD behavior. The structure of this model is very different from that needed to model chemical and drug dermal distribution in the same perfused tissue system. The implications of this difference in model structure to systemic disposition will be discussed and explored using simulated PBPK models.

S 1975 PHYSIOLOGICAL AND MATERIAL PROPERTY DETERMINANTS OF NANOMATERIAL KINETICS: A PHYSIOLOGICALLY BASED FRAMEWORK FOR NANOMATERIAL DOSIMETRY AND KINETICS.

J. G. Teeguarden[1], P. M. Hinderliter[1], B. D. Thrall[2] and J. P. Pounds[2]. [1]*Biological Monitoring and Modeling, Pacific Northwest National Laboratory, Richland, WA and* [2]*Cell Biology, Pacific Northwest National Laboratory, Richland, WA.*

This presentation will bring together current empirical knowledge on the kinetics of nanomaterials with the material property and physiological determinants of tissue uptake to develop a framework for developing physiologically motivated kinetic models for nanomaterials. The wealth of data on size and surface chemistry dependencies of nanoparticle tissue uptake and blood clearance is available in the pharmaceutical and biomedical literature as is information on mechanisms of active uptake by tissues of the reticuloendothelial system. For instance, the kinetics of nanomaterial tissue uptake is often driven by active uptake rather than traditional partitioning. Tissues such as the lymph nodes and spleen, not typically important in pharmacokinetic models of chemicals, are central to the tissue distribution of nanomaterials. Size, agglomeration state and surface charge dependencies in the tissue uptake have been reported and must be considered in kinetic modeling. The available information will be organized and integrated to establish principles for understanding and quantitatively describing nanomaterial kinetics computationally.

W 1976 ADVANCES IN TECHNOLOGY AND INCREASING ACCEPTANCE FOR ZEBRAFISH USE IN DRUG DISCOVERY.

M. J. Carvan, [1] and R. L. Tanguay[2]. [1]*Great Lakes WATER Institute, University of Wisconsin-Milwaukee, Milwaukee, WI and* [2]*Department of Environmental & Molecular Toxicology, Oregon State University, Corvallis, OR.*

Drug discovery is an increasingly expensive and inherently risky process, with high rates of attrition often at late stages of R&D primarily due to organ toxicity or safety pharmacology issues. High-profile withdrawals of drugs from the market due to unforeseen, dangerous side effects have drawn extra attention to the difficulties of predicting toxicity using traditional methods and the US FDA has also acknowledged a technology deficit in toxicity testing at preclinical stages. The zebrafish has emerged as a valuable vertebrate model for use by the pharmaceutical industry for expediting the process of prioritisation of candidate drugs for development and flagging toxicity early in the R&D process. Vast amounts of academic studies over the past three decades have shown that zebrafish have conserved genetic pathways and that the ease of their genetic manipulation has led to identification of numerous mutations that phenocopy human diseases. In addition, their small size allows high throughput screening in microtiter plates using minuscule amounts of compounds. This has consequently made the developing zebrafish embryo an attractive model vertebrate for use in safety pharmacology and drug toxicity testing. These endeavours should help reduce the cost of drug discovery, reduce the use of higher vertebrates and decrease the risk of advancing potentially toxic compounds towards

clinical testing. This symposium will focus on recent advances in zebrafish-based technologies including automation and screening of transgenics, validation of toxicity screens using this zebrafish model that show good concordance with mammals, and other novel discoveries made possible due to the zebrafish.

W 1977 ZEBRAFISH USE IN DRUG DISCOVERY.

A. J. Hill. *Biology, Summit plc, Abingdon, Oxon, United Kingdom.*

During late stage drug development high rates of attrition are commonly attributable to organ toxicity or issues with safety pharmacology demonstrating that predicting these problems using traditional methods can be difficult as well as costly. Utilizing information obtained over three decades of academic research, the zebrafish has emerged as a valuable vertebrate model for use in drug discovery for expediting the process of prioritization of candidate drugs for development and identifying toxicity. In addition their small size makes the zebrafish larvae amenable to high throughput screening in microtiter plates early in the R&D process. Such assays require very small quantities of compounds and are also amenable to automation. This heightened awareness of this model organism has consequently led to significant advances in protocol design and enhanced validation for both toxicity and safety pharmacology screens. This includes better evaluation and predictability for compounds which may produce acute toxicity and toxicity particularly at the level of the heart and liver. In particular, the importance of determining actual concentrations of compounds within the larvae rather than relying on aqueous concentrations applied to the fish to rank compounds for toxicity has been identified. Likewise, the use of LogP to estimate compound absorbance has been investigated and found to be variable; and therefore promotes the need for a method of bioanalysis. These extensions to existing zebrafish protocols will result in a wider acceptance for the zebrafish model and will reduce toxicity related attrition late in drug discovery.

W 1978 UNRAVELING ENVIRONMENTAL BIOLOGICAL INTERACTIONS USING ZEBRAFISH.

R. L. Tanguay[1, 2], L. K. Mathew[1, 2] and S. L. Harper[1, 2]. [1]*Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR and* [2]*Environmental Health Sciences Center, Oregon State University, Corvallsi, OR.*

There is an increased need for rapid, relevant and efficient in vivo testing strategies to evaluate chemical and biological interactions in order to protect and improve human and environmental health. Early developmental life stages are often uniquely sensitive to environmental insult, due in part to the enormous changes in cellular differentiation, proliferation and migration required to form the required cell types, tissues and organs. Molecular signaling underlies most of these processes. Most toxic responses result from disruption of proper molecular signaling, thus embryonic development is perhaps the ideal life stage to determine if chemicals or nanomaterials are toxic. We will discuss the tiered approach that we use to evaluate the potential toxicity of pharmaceuticals, environmental chemicals and novel engineered nanomaterials. The emphasis of this presentation, however, will be on how we exploit the inherent advantages of zebrafish to move beyond hazard identification. Using molecular and genetic approaches the zebrafish model offers unique opportunities to unravel the molecular mechanisms underlying toxic responses.

W 1979 FLUORESCENT ZEBRAFISH ASSAYS FOR DRUG DISCOVERY AND TOXICOLOGY.

P. Eimon. *Zygogen LLC, Atlanta, GA.* Sponsor: A. HILL.

Zebrafish toxicity assays will allow safety profiling to be performed early in the drug discovery process, when choices must be made between thousands of candidate compounds. Screening large numbers of compounds in zebrafish requires automation at all steps of the assay, including embryo distribution, liquid handling, imaging, and data analysis. The use of transgenic fish with fluorescent organs and tissues facilitates the automation of imaging and data analysis. For example, we have developed an in vivo zebrafish cardiotoxicity assay that utilizes a transgenic line with a fluorescent heart to automate analysis of heart rate and rhythm. These key indicators of cardiac health provide readouts that correlate well with compounds known to cause long QT syndrome (LQTS) and Torsades de Pointes (TdP). Specifically, we find that compounds associated with LQTS in humans cause bradycardia in zebrafish, while those associated with TdP cause cardiac both bradycardia and arrhythmia. Additional tissue-specific fluorescent transgenic lines open up the possibility of automated quantitative assays in other areas of toxicology, including neurotoxicity. For example, when zebrafish embryos with fluorescently labeled neurons are exposed to several common neurotoxins, significant reductions in fluorescence can be observed and quantified. Fluorescent zebrafish assays can also be combined with morpholino antisense technology to rapidly examine the function of individual gene targets and ascertain potential side effects associated with inhibitory therapeutics.

W 1980 VALIDATION OF ZEBRAFISH AS A MODEL FOR SCREENING TERATOGENICITY.

A. Gustafson[1] and A. J. Hill[2]. [1]*R&D, Safety Assessment, AstraZeneca, SE-151 85 S√É¬∂dert√É¬§lje, Sweden and* [2]*Biology, Summit plc, Abingdon, Oxon, United Kingdom.*

In recent years several assays have been designed to screen compounds for in vivo teratogenicity and embryotoxicity earlier in drug development, prior to the traditional studies performed in rats and rabbits. However, none of them have gained broad acceptance by the drug discovery industry. Toxicity assays in the zebrafish (Danio rerio) provide several advantages compared to other screens such as the EST or the WEC and these include (i) rapid organogenesis in zebrafish embryo/larvae resulting in quick assay turnover, (ii) regular and simple harvesting of embryos, (iii) small compound requirements and (iv) high content/high throughput analyses. As part of a Toxicity Consortium consisting of five pharmaceutical companies and Summit plc (formerly VASTox), 50 compounds were screened blind using the embryonic zebrafish to evaluate their potential to cause teratogenicity and embryotoxicity. Larvae were exposed to multiple concentrations of test substances at 4 hours post fertilization (sphere stage/midblastula; equivalent to 6 days in rats/rabbits) and assessed at 96 hours post fertilization (free swimming larva; equivalent to 16/18 days in rats/rabbits). Twenty eight morphological and functional endpoints of toxicity were assessed with each concentration tested and was followed by bioanalytical determination of levels of compounds in the larvae. The correlation between effects noted in the zebrafish and those observed from mammalian studies was assessed. This initial evaluation points towards the zebrafish teratogenicity and embryotoxicity test being a promising method for the early screening of lead compounds during early drug discovery.

W 1981 CHLOROTRIAZINE HERBICIDES AND THEIR COMMON DEGRADATION PRODUCTS OF CONCERN: DISPOSITION AND POTENTIAL HEALTH EFFECTS.

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Since the last atrazine risk assessment in 2002, the EPA Office of Water has discussed developing a triazine cumulative risk assessment of the regulated parent compounds and their common degradation products of concern in drinking water. The chlorotriazine herbicides metabolize/degrade to the same subset of residues, which are reported to be biologically active. These residues also occur in water as common chlorodegradates of the parent compounds. Atrazine is reported to have a short half-life in the rat (less than 1 day), and is primarily excreted in the urine. Recent studies using acute exposures and lower doses (at or lower than the current NOAEL/LOAELs of the parent compounds) have shown adverse developmental outcomes in the brain, prostate, and mammary gland of rodent models, in addition to those effects reported at higher doses. Because of the growing number of health effects following chlorotriazine exposure in mammals, analytical tools and high quality standards have been developed to measure the metabolites/degradates and their related excretory or conjugated forms. These data can be used to determine the disposition of the individual metabolites in the body fluids and affected tissues. Furthermore, the measurement of these triazine residues in rodent models have only recently been reported in mice, building on the limited pharmacokinetic information from male and virgin female rats. Post-exposure metabolite/degradate analyses in pregnant and lactating rats discovered unique patterns of analytes based on whether the exposure was a mixture of residues or the parent compound alone, as well as a dose effect. The developmental health effects and the measurement of the chlorotriazine metabolites in affected tissues or body fluids in multiple species and under varied reproductive/developmental conditions are important data that may be used for future risk assessment of these high use herbicide residues. *This abstract does not necessarily reflect EPA policy.*

W 1982 WHERE DO WE STAND ON CHLOROTRIAZINE RESIDUALS? CURRENT STATE OF THE SCIENCE AND REGULATION.

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The 1996 Safe Drinking Water Act (SDWA) Amendments requires the U.S. Environmental Protection Agency (EPA) to develop a list of unregulated contaminants that are known or anticipated to occur in drinking water and to publish this

Contaminant Candidate List (CCL) every five years. The CCL is used to determine which chemicals should be prioritized for potential regulation. Based on the 1996 SWDA Amendments, once a contaminant is regulated, EPA is required to review these regulations every six years, and determine appropriate regulation revisions. Presently the EPA Office of Water is proceeding with the 2nd "Six Year Review" process and hopes to use the new occurrence and toxicity data on some of these contaminants, like atrazine, simazine and their common chlorodegradates, in this risk assessment process. Atrazine is a systemic herbicide that acts by blocking photosynthesis. Introduced in 1958, it rapidly became the most used herbicide for weed control; however, it also became one of the most commonly occurring contaminants in drinking water and one of the highest occurring pesticides in ambient water. The EPA has been evaluating atrazine and its metabolites for many years. Although there have been successful drinking water regulations of atrazine and its analogue, simazine, no regulation is available on their primary common chlorometabolites. These chlorometabolites are also contaminants of concern in drinking water. We will discuss an overview of the assessment activities, comments provided by the Science Advisory Panel, and implications of new data. These data are very important for the Office of Water since the chlorometabolites are considered as degradation products of concern under the CCL, while atrazine and simazine, the parent compounds, are now included in the six-year review process. A decision to regulate these chlorodegradates as separate contaminants in drinking water under the CCL process, or as components of a cumulative risk for the triazines in drinking water is yet to be decided. *This abstract does not necessarily reflect EPA policy.*

W 1983 ATRAZINE AND DOPAMINE NEUROTOXICITY.

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Based on the report of altered hypothalamic catecholamines as the mechanistic basis of atrazine (ATZ) toxicity in female hormonal systems and the male reproductive tract, we hypothesized that ATZ would adversely affect brain dopamine (DA) systems. Dietary ATZ exposure of adult male rats for 6 mos produced persistent hyperactivity, and altered monoamine function and behavioral responsivity to amphetamine. Notably, ATZ decreased numbers of tyrosine hydroxylase-positive (TH+) and -negative (TH-) cells in both DA tracts. Acute i.p. ATZ exposures were used to examine potential mechanisms of this DA neurotoxicity. Acute ATZ reduced basal and potassium-evoked striatal release of DA but not its metabolites, as measured by microdialysis. It also increased the content of DA and metabolites in striatum, nucleus accumbens, midbrain and prefrontal cortex, and serum corticosterone levels 90 min after administration with effects sustained in some regions for 4 hrs. Additionally, increases in striatal DA and DOPAC release in response to the dopamine D2 receptor antagonist haloperidol following acute ATZ suggested increased TH activity and/or altered catecholamine biosynthesis. Further evaluations revealed that ATR did not change TH content in striatum, nucleus accumbens or midbrain. ATR did, however, increase TH activity in striatum, nucleus accumbens and midbrain from 90 min to 4 hrs after a single exposure, and also increased phosphorylation of TH at sites 19 and 40, reflecting an increase in the activity of this enzyme. These studies demonstrate that ATZ can produce neurotoxicity to DA systems that are critical to the mediation of movement, cognition and executive function and may act by increasing TH activity and affecting high affinity uptake and vesicular DA dynamics of DA systems. As such, ATZ may be an environmental risk factor contributing to DA system disorders, underscoring the need for further investigation of its mechanism(s) of action and corresponding assessment of its associated human health risks.

W 1984 COMPLEX PHARMACOKINETIC DISPOSITION OF ATRAZINE AND ITS CHLORINATED METABOLITES IN THE RAT AFTER ORAL ADMINISTRATION.

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Atrazine (ATRA) is extensively metabolized to chlorinated and non-chlorinated metabolites in the rat. The toxic responses observed in rodent models after ATRA exposure likely result from net target tissue exposure to total chlorinated triazines (Cl-TRIs) rather than administered ATRA. Accurate determination of the risk of Cl-TRIs to exposed human populations is limited by uncertainties in the extrapolation of the dose-response behavior of these compounds using animal toxicity data to predict responses in humans. This presentation will highlight the research pursued to reduce the uncertainties that exist regarding Cl-TRI tissue dosimetry. In vitro and in vivo studies on the pharmacokinetic disposition of ATRA and its chlorinate metabolites in the rat were used to develop a series of physiologically based pharmacokinetic (PBPK) models that describe the physiological and biochemical processes likely regulating the kinetic disposition of ATRA and its metabolites in plasma and target tissue (the brain). Processes such as complex oral absorption, plasma protein and hemoglobin reactivity, extensive oxidative metabolism in the liver and presumably intestines and systemic clearance by GST mediated by GSH conjugation and urinary elimination all contribute to limited tissue exposure to ATRA while producing high tissue concentrations of its predominant chlorinated metabolite, diaminochlorotriazine. These current models provide a more quantitative understanding of the processes that regulate Cl-TRI target tissue dosimetry and identify some key data gaps that will need to be addressed to improve the risk assessment of Cl-TRIs.

W 1985 METABOLISM AND HEALTH EFFECTS OF ATRAZINE EXPOSURE IN THE MOUSE.

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A growing number of *in vivo* toxicity studies with many environmental contaminants, including with atrazine, are conducted in mice. Because of the lack of data describing atrazine's pharmacokinetics and tissue dosimetry in the mouse, we will first present our recent findings describing the *in vivo* metabolism of atrazine in male C57BL/6 mice. Next, we will present our data pertaining to the effects of atrazine on the nervous and immune systems and correlate adverse health outcomes with actual target tissue levels of atrazine and its major mammalian metabolites. In our metabolism study, male C57BL/6 mice were orally exposed to a dose range (5-250 mg/kg) of atrazine. Plasma, tissues (liver, spleen, thymus, kidneys, and brain), urine, and fecal material were collected at various time points up to 96 h post dosing and levels of atrazine and its metabolites didealkyl atrazine (DACT), desisopropyl atrazine, desethyl atrazine, atrazine-glutathione conjugate, and atrazine-mercapturate were determined. Atrazine was rapidly metabolized in mice and DACT was the most abundant metabolite detected in the urine and plasma samples, as well as in the various tissues, including the brain. In separate studies, where C57BL/6 mice were exposed to atrazine for 14 days, detrimental effects of this herbicide on the nervous and immune systems were observed. As circulating and target tissue levels of the atrazine metabolite DACT are much greater than the level of atrazine, either (*i*) atrazine is a fairly potent neuro- and immunotoxicant, or (*ii*) some of the *in vivo* effects that we and others have observed are indirect and/or caused by its metabolite (s). [Supported by NIH P20RR017661].

W 1986 DISPOSITION AND HEALTH EFFECTS OF CHLOROTRIAZINE METABOLITES IN THE PREGNANT RAT AND HER OFFSPRING.

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Atrazine (100 mg/kg/d), as well as a mixture of atrazine and its metabolites at (0.09-8.7 mg/kg/d), causes delayed development of the mammary gland and increased inflammation of the prostate of Long Evans rat offspring exposed during late pregnancy. The mammary effects have been shown to alter weight gain of pups in the F2 generation (of F1-exposed dams), presumably because the effects on the mammary epithelium persist long after the exposure has been eliminated, leading to possible malnutrition of the young. Interestingly, control pups fostered to dams exposed to atrazine during days 17-19 of pregnancy exhibit the same delays in mammary gland development as those exposed only via placental transfer, and males have increased distribution of prostatic inflammation when exposed postnatally. These findings led to a series of studies to identify the circulating biological metabolites of the two variant exposures described here, determine the disposition of metabolites, and determine the timing of elimination of the metabolites/degradates. The simplest chlorotriazine metabolite, diaminochlorotriazine was the only one present in amniotic fluid one day after exposure. Chlorotriazine metabolites varied in the sera of rats exposed to atrazine parent vs metabolite mixture. Further, the chlorotriazine metabolites and their mercapturated, or otherwise conjugated, isoforms were present in the pregnant/lactating rat far longer than previously reported in the virgin female or male rat. It is also apparent that expressed milk contained atrazine at least 6 days after exposure ceased. Histological sections of the maternal mammary gland reveal severely dilated ducts that return to normal size when atrazine is no longer measurable in the milk. The most potent biological component of the atrazine metabolite mixture is under investigation. *This abstract does not necessarily reflect EPA policy.*

W 1987 INTERDISCIPLINARY APPROACHES FOR IMPROVING CHEMICAL HAZARD TESTING PARADIGMS.

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Governments, industry and non-governmental organizations are faced with the pressing need for evaluating tens of thousands of chemicals (e.g., high production volume chemicals, pesticidal inerts, and drinking water contaminants) for their potential to cause adverse human health and environmental effects. Traditional approaches for toxicological assessment are too labor- and cost-intensive to apply to all but a small segment of these chemicals. Thus, novel chemical prioritization programs are being actively developed with the goal of exploring alternative methods comprising in silico modeling, high-throughput screening and toxicogenomic technologies. The overall goal of these efforts is to characterize the molecules and/or biological processes that are the targets for chemical-biological interactions and can serve as predictors of adverse effects. This session presents both the scientific approaches being developed, as well as perspectives from various stakeholders on applications and promises of novel chemical prioritization methods.

W 1988 U.S., CANADIAN AND EUROPEAN UNION APPROACHES TO PRIORITIZING AND IDENTIFYING INDUSTRIAL CHEMICALS OF CONCERN.

R. A. Denison. *Environmental Defense, Washington, DC.* Sponsor: D. Dix.

Policies governing industrial chemicals all provide criteria and mechanisms to identify chemicals of concern and prioritize them for development of additional risk-relevant information through testing or other means. The approaches taken by regulatory agencies in the US, Canada and EU vary substantially in many respects, traceable in large part to differences in each jurisdiction's chemical regulatory statutes. This presentation will compare and contrast the three policies with respect to how they have sought to prioritize chemicals for further information development, and how well they have been able to identify and act to address the risks of chemicals of concern. The presentation will highlight strengths and weaknesses of the approaches, and will seek to place each approach in a broader societal context, including how each of these jurisdictions is responding to changes in the nature of concerns about health effects of chemicals and the growing calls for major reform in how we identify, assess and mitigate chemical risks.

W 1989 APPROACHES FOR PRIORITIZING AND TESTING OF CHEMICALS FOR TOXICITY.

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A tiered testing approach is generally needed for the collection of appropriate hazard information for all products, both new and existing. In the agro-chemical industry, the number of new chemical entities (NCE) advanced to the end use applications is relatively small owing to multiple filters for establishing product efficacy in laboratory settings. *In silico* and *in vitro* toxicity screening tests are often used to identify potential "red flags" during the discovery of NCEs to help in the identification of molecules that are expected to have a favorable toxicity profile. For those NCEs that are advanced further in the product development, appropriate toxicity and exposure assessments are performed for risk assessment and risk management actions. In prioritizing toxicity testing for existing or new industrial chemicals, potential exposure scenarios such as production volume and projected end uses of products should be considered. Although chemical-specific laboratory generated data has several advantages, animal welfare considerations support the use of relevant and validated computational models such as (Q)SAR and read-across methodologies in lieu of animal studies. It is anticipated that refinements to these models leading to better predictive capabilities would eventually lead to a wider application of these tools. Emerging technologies such as toxicogenomics may some day play a role in prioritization of chemicals for long-term toxicity testing such as the chronic onocogenicity and multigeneration reproductive toxicity studies. While these technologies are already contributing to our understanding of a toxicant's mode of action, their utility in predicting the results of a long-term toxicology study awaits the outcome of ongoing investigations in multiple laboratories and multi-stakeholder initiatives.

W 1990 EPA'S TOXCAST PROGRAM FOR PREDICTING HAZARD AND PRIORITIZING TOXICITY TESTING OF ENVIRONMENTAL CHEMICALS.

K. Houck. *NCCT, U.S. EPA, Research Triangle Park, NC.* Sponsor: D. Dix.

ToxCast, the United States Environmental Protection Agency's chemical prioritization research program, is developing methods for utilizing computational chemistry, bioactivity profiling and toxicogenomic data to predict potential for toxicity and prioritize limited testing resources. The proof-of-concept phase of ToxCast is focused upon chemicals with an existing, rich toxicological database in order to provide an interpretive context for the ToxCast profiling data. This set of several hundred reference chemicals represents numerous structural classes and phenotypic outcomes, including tumorigens, developmental and reproductive toxicants, neurotoxicants and immunotoxicants. The ToxCast program is evaluating chemical properties and bioactivity profiles across a broad spectrum of data domains: physical-chemical, predicted biological activities based on existing structure-activity models, biochemical properties based on high-throughput screening assays, cell-based phenotypic assays, and genomic analyses of cells. These data are being generated through a series of external contracts, and through collaborations within EPA and with the National Institutes of Health Chemical Genomics Center. The resulting multidimensional dataset requires appropriate computational methods for integrating various chemical, biological and toxicological data into profiles and models predicting toxicity. This requirement is being met by the ACToR system (Aggregated Computational Toxicology Resource) to provide a repository and context for data from the ToxCast program.
This work was reviewed by EPA and approved for publication but does not necessarily reflect official Agency policy.

W 1991 MODELING TOXICITY FROM HIGH THROUGHPUT SCREENING DATA ON ENVIRONMENTAL CHEMICALS.

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To develop efficient approaches for rapid evaluation of chemical toxicity and human health risk of environmental compounds, the National Toxicology Program (NTP) in collaboration with the National Center for Chemical Genomics has initiated a project on high throughput screening (HTS) of environmental chemicals. The first HTS results for a set of 1,408 compounds tested for their effects on cell viability in 6 different cell lines have recently become available via PubChem. We have explored these data in terms of their utility for predicting adverse health effects of the environmental agents. Initially, the classification k Nearest Neighbor (kNN) Quantitative Structure-Activity Relationship (QSAR) modeling method was applied to the HTS data only, for a curated dataset of 557 compounds. The resulting models had prediction accuracies for training, test (containing 400 compounds together), and external validation (157 compounds) sets as high as 79%, 79%, and 84%, respectively. We then asked if HTS results could be of value in predicting rodent carcinogenicity. We identified 383 compounds for which data were available from both the Berkeley Carcinogenic Potency Database and NTP-HTS studies. We found that compounds classified by HTS as "actives" in at least one cell line were likely to be rodent carcinogens (sensitivity 77%); however, HTS "inactives" are far less informative (specificity 46%). Using chemical descriptors only, kNN QSAR modeling resulted in 62.3% prediction accuracy for rodent carcinogenicity applied to this data set. Importantly, the prediction accuracy of the model was significantly improved (72.7%) when chemical descriptors were augmented by HTS data, which were regarded as biological descriptors. Our studies suggest that combining NTP HTS profiles with conventional chemical descriptors could considerably improve the predictive power of computational approaches in toxicology.

W 1992 REPLACEMENT OF ANIMAL TESTING WITH *IN VITRO* ALTERNATIVES FOR CHEMICAL SAFETY SCREENING THROUGH TOXICOGENOMICS.

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Major changes in chemical safety management in the European Union necessitate the development of new risk assessment paradigms that may not rely so heavily on data generated by animal experiments. The EU Chemicals Regulation (REACH) will dramatically increase the need for generating new types of chemical safety data. In addition, other regulations are being considered by the European Commission that would greatly expand the scope of safety/benefit testing of food ingredients and other products. In this context, genomics technologies are considered, f.i. by REACH, of potential in that they may generate high-dimensional data on molecular mechanisms underlying toxicant mode of action and dose-response. The Netherlands Toxicogenomics Centre is developing toxicogenomics-based in vitro assays using (human) cell lines and primary cells, which consider complex multidimensional relationships between exposure, biological response, and human health outcomes relating to chemical carcinogenesis, immunotoxicity and reproductive toxicity.

1993 IMMUNOLOCALIZATION OF GSTA4-4 IN ENDOTHELIAL CELLS AND POSSIBLE ROLE IN PROTECTION AGAINST APOPTOSIS AND ATHEROSCLEROSIS.

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4-Hydroxynonenal (4-HNE), the end product of lipid peroxidation are toxic and may trigger cell apoptosis and cell death. The compound are mainly metabolized by alpha class glutathione S-transferase A4-4 (GSTA4-4), such as mGSTA4-4 or hGSTA4-4 expressed in mouse and human tissues respectively. Our previous work on MS1 endothelial cells transfected with mGSTA4 cDNA comfirmed that mGSTA4-4 played a critical role in protection of endothelial cells against apoptosis through JNK cell signaling pathway.

The introcellular localization of mGSTA4-4 in transfected MS1 endothelial cells and possible protective role against cell apoptosis was investigated by electronic microscope or confocal immunofluerenscent method, 4-HNE and hGSTA4-4 expression in the human atherosclerotic plaques at different stages were also studied by immunohistochemical staining in this work. Western blot of cytoplasmic and mitochondrial frational protein was carried out for Bcl 2 and cytochrome c assay.

mGSTA4-4 and 4-HNE were found colocalized in cytoplasm, immuno-electronic microscope comfirmed that mGSTA4-4 localized in cell vesicle close to cell membrane. The early human atherosclerotic plaque showed less 4-HNE expression while hGSTA4-4 was induced in endothelial cytoplasm. Bcl 2 was upregulated and cytocrome c release from mitochondria to cytoplasm was blocked in mGSTA4-transfected cells compared to control cells under oxidative stress.

This data suggest that GSTA4-4 preferentially localized in cytoplasm close to membrane that may contain more substrates like 4-HNE, make it easy to play metabolizing role agains apoptosis.

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1994 GONIOTHALAMIN INDUCES CORONARY ARTERY SMOOTH MUSCLE CELLS APOPTOSIS IN P53 DEPENDENT AND NQO1 INDEPENDENT MANNER.

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In stent stenosis (ISS) remains a major obstacle to the success of coronary angioplasty. The pathogenesis of ISS involves aberrant proliferation of coronary artery smooth muscle cells (CASMCs) in response to vascular injury. Apoptosis induction and inhibition of proliferation are effective ways to prevent ISS. Goniothalamin (GN), a styryllactone derived from natural product has been shown to induce apoptosis in various cell lines including CASMCs. We have recently demonstrated that GN induces DNA damage leading to CASMCs apoptosis. In this study, we have further investigated the molecular mechanisms of GN induced apoptosis. Our results exhibited an elevation of p53 in CASMCs as early as 2 hours after GN treatment. We further investigated whether NADP(H): quinone oxidoreductase 1 (NQO1), a stabilizing protein for p53 is concomitantly being up regulated. However, we found that p53 elevation in GN treated CASMCs did not correlate with sustained NQO1 protein expression and activity. In agreement to the role of p53 induced apoptosis, GN treated CASMCs resulted in a time dependent increase in caspase-2 activity. These data provide insight into the mechanisms of GN induced apoptosis which may have importance in the development of drug eluting stents.

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1995 PARAQUAT INDUCED APOPTOSIS IN RAT PLEURAL MESOTHELIAL CELLS (RPMC) INVOLVES RELEASE OF CYTOCHROME C AND CASPASE-9 ACTIVATION.

K. H. Bijlani, H. R. Sukhija, J. O. Cantor and J. M. Cerreta. *PHS, St. John's University College of Pharmacy and Allied Health Professions, Queens, NY.* Sponsor: L. Trombetta.

Paraquat is a widely used herbicide. Due to structural similarity of paraquat with lung diamines and polyamines, paraquat accumulates in this organ and induces apoptosis. The current study was under taken to determine the molecular events that lead to apoptosis in RPMC treated with paraquat. RPMC were cultured in Ham's F-12 media supplemented with FBS, glutamine and antibiotics in an atmosphere of 5% CO_2 at 37° C. Cultures were exposed to paraquat (25µM) alone or in combination with either cyclosporin A (10µM)or Z-LEHD-FMK (10µM). Cell viability was determined using a MTT assay; Caspase-3 activity and TUNEL assay were used to measure paraquat induced apoptosis in cultured cells. There was a significant decrease in cell viability of cultures treated with 25µM paraquat for 24 hrs versus to the control cultures. Caspase- 3 activity and the number of TUNEL positive cells were significantly elevated in treated cultures compared to the control. Cytosolic cytochrome C and caspase-9 activity were elevated (91%,p<0.01) (305%,p<0.01), respectively in treated cultures versus controls. The following reductions were seen in cultures co-treated with paraquat and Z-LEHD-FMK compared to cultures treated with paraquat alone: Cytotoxicity (23%,p<0.05), the number of TUNEL positive cells (91%,p<0.001), caspase-9 activity (80%,p<0.001) and caspase-3 activity (85%, p<0.001). The following reductions were seen in cultures co-treated with paraquat and cyclosporin A compared to cultures treated with paraquat alone: Cytotoxicity (24%,p<0.01), TUNEL positive cells (93%, p<0.001), caspase-9 (73%,p<0.001) and caspase-3 activity (72%, p<0.001). Cytochrome C levels were reduced in cultures co-treated with paraquat and cyclosporin A compared to those treated with paraquat alone. Such results indicate that paraquat induces apoptosis in RPMC by activation of caspase-9 via a mitochondrial pathway and such paraquat induced apoptosis is attenuated by co-treating cultures with Z-LEHD-FMK or Cyclosporin A.

1996 A ROLE FOR CASPASE 8 IN AMIODARONE INDUCED APOPTOSIS IN RAT PLEURAL MESOTHELIAL CELLS (RPMC).

R. Shah, J. O. Cantor and J. M. Cerreta. *PHS, St. John's University College of Pharmacy and Allied Health Professions, Queens, NY.* Sponsor: L. Trombetta.

Amiodarone is a diiodinated benzofuran derivative with class III antiarrhythmic activity. Patients on amiodarone therapy may develop pulmonary fibrosis. Such fibrosis may be caused by apoptosis of the lung mesothelial cells, however, the mechanism of amiodarone induced apoptosis is unclear. Cells were cultured in Ham's F-12 Nutrient Mixture supplemented with fetal bovine serum, glutamine, antibiotics in 5% CO_2 balance air. To study the amiodarone induced apoptotic pathway, a caspase-8 specific inhibitor, Z-IETD-FMK, was used and cytochrome C was measured following treatment. Cultures treated with amiodarone (45µg/ml) had increase in cytotoxicity (50%, p<0.001), in caspase 3 activity (352%, p<0.001) and in TUNEL positive cells (247%, p>0.001). Co-treatment of cultures with amiodarone (45µg/ml) and Z-IETD-FMK (10µM) resulted in an increased (60%, p<0.05) viability, a decrease in caspase 3 activity (129%, p<0.001) and a decrease (116%, p<0.001) in TUNEL positive cells as compared to cultures treated with amiodarone alone. Cytochrome C levels in the cytoplasm were elevated (217%, p<0.01) when cultures were exposed to amiodarone (45µg/ml), but declined (59%, p<0.01) in cultures co-treated with amiodarone (45µg/ml) and Z-IETD-FMK (10µM). Phase contrast microscopy of control cultures revealed a monolayer of epithelial-like cells with polygonal shapes. Microscopy of cultures treated with amiodarone exhibited reduced numbers of shrunken, distorted cells with many dead floaters. Phase contrast microscopy of cultures co-treated with amiodarone and Z-IETD-FMK resembled the controls. Data from our laboratory and the current study clearly implicate the mitochondrial pathway for the amiodarone induced apoptosis. However, it has been shown that blocking of cytochrome C release does not completely ameliorate such apoptosis. The reduction in apoptosis by the caspase 8 specific inhibitor, Z-IETD-FMK, suggests that both the intrinsic and extrinsic pathway may be functioning in amiodarone toxicity.

1997 Z-VAD-FMK INHIBITS AMIODARONE INDUCED APOPTOSIS IN RAT PLEURAL MESOTHELIAL CELLS (RPMC).

A. Datwani, J. M. Cerreta and J. O. Cantor. *PHS, St. John's University College of Pharmacy and Allied Health Professions, Queens, NY.* Sponsor: L. Trombetta.

The clinical efficacy of amiodarone is limited due to its potential to cause pulmonary toxicity. It is hypothesized that the amiodarone toxicity is initiated in part through induction of the apoptosis pathway; however, the precise molecular events that cause such toxicity remain obscure. The current study was carried out to examine the pathway of amiodarone induced apoptosis in RPMC. Cells were cultured in Ham's F-12 Nutrient Mixture supplemented with fetal bovine serum, glutamine, and antibiotics in a humidified 5% CO_2 balanced air. Cell viability was determined by MTT cytotoxicity while caspase 3 activity and TUNEL assay were used as markers of apoptosis. Treatment of the cells with amiodarone (50µg/ml) caused an increase in cytotoxicity (50%, p<0.001), an increase in caspase 3 activity (256%, P < 0.001) and an increase in TUNEL positive cells (585%, p<0.001). Co-treatment of cultures with amiodarone (50µg/ml) and Z-VAD-FMK (4µg/ml) increased cell viability (142%, p<0.05), decreased caspase 3 activity (41%, p <0.001) and reduced

the number of TUNEL positive cells (45%, P< 0.001) as compared to cultures treated with amiodarone alone. Treating RPMC's with amiodarone caused a significant increase (134%, p< 0.001) in the levels of the caspase-9 activity and co-treatment of cells with amiodarone (50µg/ml) and Z-VAD-FMK (4µg/ml) reduced (46%, p<0.001) such enzyme levels. Amiodarone also increased levels cytoplasmic cytochrome C (197%, p<0.001) levels as compared to controls. The above results suggest that amiodarone induce cytotoxicity leads to increase in apoptosis as indicated by elevated caspase 3 activity and by increase in TUNEL positive cells. Such increases were significantly reduced when cultures were co-treated with Z-VAD-FMK. Increases in levels of cytochrome C and caspase 9 suggest that amiodarone induced apoptosis in RPMC is following the mitochondrial pathway.

1998 Z LEHD FMK AND CYCLOSPORIN A INHIBIT APOPTOSIS INDUCED BY CADMIUM CHLORIDE IN AT PLEURAL MESOTHELIAL CELLS (RPMC).

H. R. Sukhija, K. H. Biljani, J. O. Cantor and J. M. Cerreta. *PHS, St. John's University College of Pharmacy and Allied Health Professions, Queens, NY.* Sponsor: L. Trombetta.

Cadmium is a toxic metal found in workplaces, as a food contaminant and a component of cigarette smoke. Cadmium can damage organs such as the lungs, however, the mechanism by which such injury is induced is unclear. The current study investigates cellular events in cadmium induced apoptosis. RPMC were cultured in Ham's F-12 media with FBS, glutamine, and antibiotics in an atmosphere of 5% CO_2 at 37° C. Cultures treated with cadmium (15µM) had significant increase in cytotoxicity as measured by MTT Assay, in caspase 3 activity and in TUNEL positive cells as compared to controls. Cultures co-treated with cadmium (15µM) and cyclosporin A (1µg/ml) had a significant decrease in cytotoxicity, in caspase 3 activity (87.6%, p< 0.01) and in TUNEL positive cells (81.678%; p< 0.001) as compared to cadmium controls. Co-treatment of cultures with cadmium (15µM) and Z-LEHD-FMK (10µg/ml) significantly decreased cytotoxicity, caspase 3 activity (91.4 %; p<0.01) and TUNEL positive cells (76.694%; p<0.001) as compared to cadmium treated cultures. Cultures treated with cadmium (15µM) had a significant increase in caspase 9 activity while those co-treated with cadmium and cyclosporin A (1µg/ml) or Z-LEHD-FMK (10µg/ml) had a reduction in such activity by 69.29%; p<0.001 and 73.085%; p< 0.001 respectively as compared to cadmium treated cultures. Cultures treated with cadmium (15µM) had an increase in cytoplasmic cytochrome C levels while those co-treated with cadmium and cyclosporin A (1µg/ml) had a decrease (44.16%; p <0.001) in cytochrome C levels as compared to cadmium controls. Z-LEHD-FMK and cyclosporin A significantly reduced apoptosis. In addition, the reduction in cytoplasmic cytochrome C levels in conjunction with the reduction in caspase 9 activity suggest that apoptosis is occurring through the mitochondrial dependent pathway.

1999 CHOLESTEROL SECOALDEHYDE INDUCES APOPTOSIS IN J774 MACROPHAGES *VIA* MITOCHONDRIAL PATHWAY BUT NOT INVOLVING REACTIVE OXYGEN SPECIES AS MEDIATORS.

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Cholesterol secoaldehyde (ChSeco), a putative product of cholesterol oxidation by ozone or singlet oxygen, has increasingly been recognized to play a role in the pathogenesis of atherosclerosis and Alzheimer's diseases. The cytotoxic effect of this oxysterol towards murine neuronal cells and rat cardiomyocytes has been studied in detail. In the present study, we examined the cytotoxic responses in mouse macrophages (J744) exposed to ChSeco. Disruptions of mitochondrial membrane potential (64 ± 5.5%; mean ± SD, n = 3), increase in the release of cytochrome *C* (8.8 ± 0.84 ng/mL; mean ± SD, n = 3) in the cytosol, and DNA fragmentation (*ca.* 5-fold), all indicative of apoptotic cell death, were observed in response to ChSeco treatments. The apoptotic cell death in J774 macrophages treated with ChSeco was also confirmed by acridine orange and ethidium bromide dual staining. However, unlike the case with cardiomyocytes and neuronal cells, the apoptotic process in these immune cells was not mediated by reactive oxygen species, as indicated by a minimal or no increase in the hydrolysis and subsequent oxidation of 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescene diacetate (CM-H_2DCFDA) to 5-(and-6)-chloromethyl-2',7'-dichlorofluorescene (CM-DCF). The mechanism of ChSeco-induced apoptosis in J774 macrophage is not clear; however, the observed loss in mitochondrial membrane potential and increased cytochrome *C* level in the cytosol indicate the involvement of an intrinsic pathway of apoptosis. [Funding support from NSF (grant number HRD 0450375) and NIH (grant number P20 RR 16456) to RMU is acknowledged; Corresponding author, e-mail: rao_uppu@subr.edu.]

2000 OXIDATIVE LIPIDOMICS OF HYPEROXIC ACUTE LUNG INJURY.

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Hyperoxic acute lung injury (HALI) has been long associated with the emergence of oxidative stress, particularly lipid peroxidation, and disruption of pulmonary endothelium. An assembly of interacting factors leads to triggering apoptosis of pulmonary endothelium during development of HALI. Signaling roles of phospholipid (PL) oxidation products in HALI-associated endothelial apoptosis has not been studied. We employed oxidative lipidomics approach to identify individual molecular species of polyunsaturated PLs involved in apoptosis-associated peroxidation process. By using a combination of ESI-MS and fluorescence HPLC we established that pulmonary lipids undergo non-random peroxidation during HALI: two anionic PLs – mitochondria-specific cardiolipin and plasma membrane phosphatidylserine – are the major oxidized PLs while more abundant PLs such as phosphatidylcholine (PC) and phosphatidylethanolamine (PE) – remain non-oxidized. Using sheep pulmonary endothelial cells (SPAEC) treated with lipopolysacharaide (LPS) (100 ng/ml, 4h at 37oC) as an in vitro model, we established a marked activation of caspases 3/7 as a biomarker of execution of apoptotic program. Similar to hyperoxic lung injury in vivo, ESI-MS analysis revealed the formation of several oxygenation products in the same two anionic PLs – CL and PS - in SPAEC challenged with LPS. Detailed MS analysis of CL oxidation products - doubly-charged ions at m/z 729 – identified its fatty acid residue with hydroperoxide moiety at m/z 311 (C18:2+OOH) along with 255 (C16:0), 279 (C18:2), and 282 (C18:0). PS oxidation products were mostly represented by its species containing C22:6-OOH. Two most abundant PLs, PC and PE, did not reveal any oxidation products in spite of the presence of polyunsaturated molecular species. We speculate that cyt c driven oxidation of CL and PS is associated with the execution of apoptosis in pulmonary endothelial cells thus contributing to endothelial cell dysfunction in HALI. Support: HL070807.

2001 3-METHYLINDOLE INDUCED DNA DAMAGE PARTITIONS BETWEEN REPAIR OR APOPTOSIS IN HUMAN BRONCHIAL EPITHELIAL CELLS, DEPENDING ON DOSAGE.

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Bioactivation of 3-methylindole (3MI)by pulmonary cytochrome P450 enzymes is directly associated with formation of DNA adducts and single strand breaks in the DNA of cultured human lung cells. We present evidence that this damage can be repaired with time, given a low level of exposure, and that higher levels of exposure and adduct formation lead to apoptosis. Cells treated with 10 µM 3MI for 4 hours show extensive DNA damage when measured by the comet assay, but if washed and allowed to recover for 20 additional hours, show no more damage than control cells. Cells treated with 10 µM 3MI for 24 hours show no greater increase in DNA damage above what is seen at 4 hours, implying an equilibrium between damage and repair. Poly ADP ribose polymerase (PARP) functions to repair single strand breaks in DNA. Pretreatment with an inhibitor of PARP nearly double the DNA damage produced by 5 µM 3MI. Nuclear localization of p53 is seen in 25 % of lung cells when treated with 100 nM 3MI for 1 hour and 60% with 50 µM 3MI. In normal cells, p53 signaling can lead to cell cycle arrest and up regulation of DNA repair enzymes or caspase-mediated apoptosis if DNA damage is beyond repair. By both Annexin V binding and DNA fragmentation, we find that 3MI begins to induce apoptosis at 10 µM in normal human lung cell culture and that apoptosis becomes extensive at 100 µM, similar to the extent seen with 10 µM doxorubicin, a potent anticancer drug. Xenobiotic-induced mutation occurs within the dose range where the genotoxic substance damages DNA enough to require repair but does not do so to the extent of killing the cell by inducing apoptosis. Our findings indicate that there is a dosage window in which 3MI can cause extensive DNA damage and repair without triggering apoptotic defenses, further supporting the hypothesis that inhaled 3MI from cigarette smoke is a potent lung-specific carcinogen. *Support: HL13645 from The National Heart, Lung and Blood Institute.*

2002 THE MECHANISM BY WHICH CANNABIDIOL, A PLANT-DERIVED CANNABINOID, SUPPRESSES IMMUNE FUNCTION INVOLVES SUPPRESSION OF NUCLEAR FACTOR OF ACTIVATED T CELLS (NFAT) TRANSCRIPTIONAL ACTIVATION, BUT NOT SUPPRESSION OF NFATC1 OR NFATC2 mRNA EXPRESSION.

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Cannabidiol (CBD) is one of more than 60 cannabinoid compounds found in marijuana. CBD and other cannabinoid compounds exhibit immunomodulatory effects. A major target of CBD is the transcription factor, nuclear factor of activated T cells (NFAT). NFAT represents a family of transcription factors that are critically important in the transcriptional regulation of several cytokine genes, including interferon-γ (INF-γ). It has been demonstrated that CBD suppressed NFAT transcriptional activation in response to phorbol ester and calcium ionophore (PMA/Io) in Jurkat T cells transfected with an NFAT reporter construct. Thus, the purpose of these studies was to further characterize the effects of CBD on IFN-γ. Using splenocyte preparations, it was determined that CBD suppressed PMA/Io- and anti-CD3 plus anti-CD28-stimulated IFN-γ production, suggesting that T cells indeed are a sensitive target of suppression by CBD. However, using splenocytes derived from athymic nude mice, CBD also suppressed PMA/Io-stimulated IFN-γ production, suggesting that CBD also targets other IFN-γ-producing cells, such as macrophages or NK cells. In addition, CBD suppressed PMA/Io-stimulated IFN-γ mRNA expression, which correlates with CBD-induced suppression of NFAT transcriptional activity. Finally, CBD had no effect on the mRNA expression of either NFATc1 or NFATc2, the two predominant NFAT isoforms in T cells, suggesting that the effects of CBD on NFAT likely occur through either post-translational mechanisms, or through suppression of signaling events upstream of NFAT. (Supported in part by NIH DA07908)

2003 ATTENUATION OF EXPERIMENTAL AUTOIMMUNE HEPATITIS BY EXOGENOUS AND ENDOGENOUS CANNABINOIDS: INVOLVEMENT OF REGULATORY T CELLS.

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Immune-mediated inflammatory liver diseases including autoimmune hepatitis are a major health problem worldwide. Cananbinoids effectively modulate immune cell function and have shown therapeutic potential in treating inflammatory diseases. We sought to investigate the immunomodulatory effects of exogenous and endogenous cannabinoids in a murine model of T cell-mediated hepatitis induced by plant lectin concanavalin A (ConA). Intraperitoneal administration of Δ^9-tetrahydrocannabinol (THC) after ConA challenge inhibited hepatitis as shown by significant decrease in liver enzyme aspartate transaminase and reduced tissue injury as indicated by liver histological examination. Furthermore, THC treatment resulted in significant suppression of crucial inflammatory cytokines (IFN-γ, TNF-α, IL-1, IL-6, IL-17) and chemokines (GM-CSF, G-CSF, KC, MIP-1α, RANTES). Interestingly, THC treatment in ConA-injected mice led to significant increase in absolute number $Foxp3^+$ T regulatory cells in liver. Select cannabinoid receptor (CB1 and CB2) agonists (ACEA and JWH-133, respectively) did not block hepatitis either independently or in combination. However, CB1/CB2 mixed agonists (CP55,940 and WIN55212) attenuated hepatitis similar to THC. Moreover, the modulatory effect of THC was reversed by CB1(AM251) or CB2 (SR144528) antagonists. Endogenous cannabinoid anandamide was able to significantly reduce hepatitis. Also, deficiency or inhibition of endocannabinoid hydrolyzing enzyme, fatty acid amide hydrolase (FAAH), which leads to increased levels of endocannabinoids, resulted in decreased liver injury upon ConA challenge. Our data demonstrate that targeting cannabinoid receptors using cannabinoids and use of FAAH inhibitors constitute novel therapeutic modalities to treat inflammatory liver diseases. (Grant support: NIH R01AI053703, R01ES09098, R01 AI058300, R01DA016545, R01HL058641, P01AT003961).

2004 C-FLIP PLAYS IMPORTANT ROLE IN TCDD (2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN)-INDUCED APOPTOSIS IN PRIMARY T CELLS AND ESTABLISHED T CELL LINE *IN VITRO*.

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While the toxic effects of TCDD on T cells in vivo have been well characterized, attempts to reproduce these findings in vitro have not been successful. In the present study, we examined TCDD-induced apoptosis in T cell in vitro using mouse primary T cells and an established T lymphoma cell line. We cultured primary T cells from mice (C57BL/6) in the absence or presence of mitogen (ConA) and mature syngeneic dendritic cells for 24 hrs and then treated them with 10-1000 nM of TCDD. We performed similar studies with EL-4 cells in the absence or presence of ConA. We observed TCDD-induced apoptosis in primary T cells only following activation with ConA or mature dendritic cells and in ConA-activated EL-4 cells. TCDD failed to induce apoptosis in primary T cells in the absence of dendritic cells or ConA. Analysis of why non-activated T cells are more resistant while activated T cells are sensitive to TCDD-induced apoptosis revealed that TCDD treatment of activated but not non-activated T cells led to upregulation of AhR, Fas, and FasL and down-regulation of c-FLIP, an inhibitor of apoptosis. We also observed cleavage of caspase 3, caspase 8, and caspase 9 in both activated T cells and EL4 cells upon TCDD exposure. Moreover, down-regulation of c-FLIP using siRNA in non-activated T cells made them sensitive to TCDD-induced apoptosis. Taken together, our data demonstrate that T cell activation or contact with mature dendritic cells in the presence of TCDD leads to upregulation of AhR, Fas, and FasL and significant downregulation of c-FLIP, which plays a critical role in triggering apoptosis (This work was supported in part by National Institutes of Health Grants R01 ES09098, R01 DA016545, R01 AI058300, R01 AI053703, R01 HL058641, R21 DA014885, ACS Institutional Research Grant, and JD Williams Research Foundation Grant).

2005 INORGANIC MERCURY MODULATES CD95/FAS DEPENDENT CROSS-REGULATION OF T-CELL ANTIGEN RECEPTOR SIGNAL TRANSDUCTION.

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Mercury (Hg) is a potent immunomodulator that affects lymphocyte signal transduction, and contributes to autoimmunity. Recent studies suggest cross-regulation between T-cell antigen receptor (TCR) and death receptor (CD95) signaling pathways. This cross-talk, represents a critical step of immune homeostasis. We have previously demonstrated that low levels of inorganic Hg attenuate CD95 dependent apoptosis as well as TCR signaling. We now test the hypothesis that Hg exposure alters the threshold of activation following CD95 receptor ligation in human T-cells. Human T-cells were stimulated with mouse anti-huCD95 in the presence or absence of $HgCl_2$ followed by single cell analysis of caspase 8, ERK1/2, and Ca^{+2} flux. A fluorogenic assay for measuring caspase 8 activity revealed that the threshold for caspase 8 activation in Hg-exposed T-cells was increased by nearly 100 fold. Specifically, the amount of caspase 8 activity following 2.5 ng/ml anti-CD95 in control T-cells was equivalent to the amount following 250 ng/ml anti-CD95 in Hg-treated T-cells. TCR dependent ERK1/2 phosphorylation was found to be reduced by Hg exposure, however, pre-incubation of cells with anti-CD95 did not appear to cause any additional modulation. In contrast, TCR dependent Ca^{+2} flux was highly dependent on cross regulation by CD95. Specifically, greater than 90% of control treated cells responded to TCR stimuli by showing elevated intracellular Ca^{+2} levels, compared with only 10% of cells pretreated with anti-CD95. Moreover, while Hg reduced the TCR dependent Ca^{+2} flux, it also partially blocked the CD95 dependent cross-inhibition (nearly 70% of cells pretreated with anti-CD95 + Hg responded to TCR stimulation). Taken together, these data support the hypothesis that Hg modulates CD95 dependent cross-regulation of TCR signal transduction. These data could have profound implications for establishment of self tolerance and autoimmunity in genetically predisposed and/or Hg-exposed populations. Supported by ES12403

2006 EX-VIVO CHARACTERIZATION OF T-REGULATORY TYPE CD4+ T CELLS IN MICE EXPOSED TO TCDD.

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The mechanisms responsible for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced immunosuppression are not well understood. Mice exposed to TCDD during an acute B6-into-B6D2F1 graft-vs.-host response do not develop disease. This resistance is dependent on aryl hydrocarbon receptor (AhR) expression in the donor T cells and correlates with an increase in donor CD4+ T cells that share characteristics with T-regulatory cells (T-regs) including high CD25 expression and suppressive function in vitro. The purpose of this study was to further characterize these T-reg-like cells (TCDD-CD4+ cells) by comparing and contrasting them with both natural T-regs and donor CD4+ cells isolated from vehicle-treated mice. Cellular anergy, suppressive function and cytokine production were examined in culture for the different cell types. Results show that TCDD-CD4+ cells are not anergic in culture despite a lack of IL-2 production. TCDD-CD4+ cells secrete significant amounts of IL-10, but do not secrete significant amounts of other Th1, Th2, or

Th17-related cytokines. Similar to natural T-regs, the suppressive function utilized by TCDD-CD4+ cells involves suppression of IL-2 expression by naïve T cells. Specific gene expression changes in TCDD-CD4+ cells as well as the enhanced phosphorylation state of STAT-4 together suggest the IL-12 signaling pathway plays a role in their generation and/or function. Though TCDD-CD4+ cells share some characteristics with natural T-regs, they have unique regulatory activity. The generation of CD4+ T-reg-like cells as a result of activation of the AHR by TCDD may represent a novel pathway for T-reg induction. Supported by NIEHS grants P01ES00040 and P30ES00020.

2007 SUPPRESSION OF T CELL CO-STIMULATOR ICOS BY DELTA-9-TETRAHYDROCANNABINOL.

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Binding of antigen to the T cell receptor (TCR) on mature T cells initiates cellular activation. However, the optimal activation and differentiation of T cells also requires a specific group of surface molecules termed T cell co-stimulators. Inducible co-stimulator (ICOS), a prototypic T cell co-stimulator, is induced on activated T cells. It is now well established that ICOS regulates T cell activation and helper T cell differentiation, and is principally involved in humoral immune responses as evidenced in ICOS knockout mice which are severely immuno-compromised. Previous work has established that the plant derived cannabinoid, delta-9-tetrahydrocannabinol (Δ^9-THC), is immunomodulatory. In particular, this laboratory has demonstrated that Δ^9-THC suppresses T cell-dependent but not polyclonal LPS-induced humoral immune responses. In light of an emerging role of ICOS in T cell-mediated immunity, the objective of this study was to investigate the effect of Δ^9-THC on ICOS expression in activated T cells. Using real-time PCR, ICOS mRNA levels were found to be induced by phorbol ester (PMA) plus ionomycin (Io) activation in primary splenocytes derived from C57BL/6 mice and was attenuated by Δ^9-THC in a concentration dependent manner. Similar results were obtained in the mouse T cell line EL4.IL-2. To confirm our results, flow cytometry was used to characterize the effect of Δ^9-THC on ICOS expression on the T cell surface. Anti-CD3/CD28 monoclonal antibodies targeting TCR and CD28 induced ICOS expression on CD4+ splenic T cells, while pretreatment with Δ^9-THC suppressed this induction in a time and concentration dependent manner. Collectively, these findings suggest that Δ^9-THC suppresses ICOS expression in activated T cells and likely contributes to suppression of T cell activation and differentiation as well as to the suppression of T cell-dependent humoral immune responses. (Supported in part by NIH DA07908)

2008 CONTAMINANTS AND A GLOBILIZED FOOD SUPPLY.

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A number of natural and anthropogenic contaminants that may exhibit adverse health effects, including neurodevelopmental, have been found in the food supply. With the food supply becoming more globalized the public health consequences at a national level have become of paramount importance. This is particularly the case for some contaminants like lead where US dietary exposures have been dramatically reduced since the 1970's, but with the introduction of new foods or sources have raised concerns about the negative impact on these reductions. The safety/risk assessment process and standards that are used to evaluate the public health significance of the presence of dietary contaminants, no matter what their origin, are determined by statutory requirements and the scientific knowledge (exposure, hazard and dose response) required to meet those standards. The FD&C Act specifically mandates a) the types of food-related substances and associated adverse health effects; b) the scientific standards of safety/risk; and c) the assignment of burden of proof required to achieve compliance with these standards. The categories of food substances (additives versus contaminants) vary in their safety/risk objectives from zero/negligible risk to significant probability of harm. In order to monitor dietary changes, particularly in a globalized food supply, the FDA develops residue data for estimating changes in dietary exposure of consumers to these food borne contaminants. A recent example of such a case was the introduction of melamine into the food supply. The FDA's annual market basket survey, the Total Diet Study, along with focused food surveys, provide this critical exposure information. The available toxicologic/epidemiologic testing and does-response assessment methodologies utilized to uncover the neurotoxic/developmental properties of food-borne substances varies considerably. Various regulatory/control procedures are available to maximize public health protection by assuring that the public is either not exposed or exposure is minimized to levels that pose negligible risk of neurodevelopmental effects.

2009 RISK ASSESSMENT OF ACRYLAMIDE IN FOOD CONTACT MATERIALS.

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The discovery of acrylamide (AA) in 2002 in many common cooked starchy foods drew increased focus on the potential risks of dietary exposure to AA.. AA has been demonstrated to be neurotoxic at very high doses in accidental worker exposures and in laboratory animals at doses of about 5 mg/kg bw/day. AA is also considered to be carcinogenic in laboratory animals by a number of organizations, e.g., IARC, US EPA. The neurotoxic and carcinogenic potential of AA has presented regulatory problems for public health organizations, particularly at levels of exposure that arise from the diet. The Food and Drug Administration (FDA) first dealt with AA in 1995 as part of the food additive approval process. AA had been found to be a contaminant of copolymers, which were intended for use in various food contact substances. Dietary exposure to AA was estimated based on migration from food contact materials, such as paper coating for dried food, and consumption of these foods. Exposures were estimated to range from 2 x 10-9 to 2 x 10-6 mg/kg bw/day. In contrast, in 2004, FDA estimated current mean US exposure to AA in the diet from the top 20 AA-containing foods, e.g., French fries, potato chips, brewed coffee, to be 4 x 10-4 mg/kg bw/day. This exposure is several orders of magnitude higher than the estimated exposure to AA from food contact materials. FDA evaluated the carcinogenic upper-bound lifetime risk from AA exposure based on a two-year rat drinking water bioassay (AA doses 0, 0.1, 0.1, 0.5 and 2.0 mg/kg bw/day) Statistically significant increases in tumors were observed at the following sites: thyroid and testes (male) and mammary, central nervous system and uterus (female). Upper bound lifetime carcinogenic risks from the AA exposures from food contact substances were estimated to range from 2 x 10-9 to 1 x 10-6 and were viewed by FDA as not posing a significant risk to human health. AA exposures from the diet will be evaluated when the NCTR bioassays of AA are completed in mid-2008; FDA's continues to advise the public to consume a healthy diet high in fruits and vegetables.

2010 ENVIRONMENTAL FATE OF MEDICINAL PRODUCTS: REQUIREMENTS AND CASE STUDIES.

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The European guidance specifying the environmental testing requirements for human medicinal products (EMEA/CHMP/SWP/44700) was formally adopted by the CHMP on the 1 June 2006, and came into effect from 1 December 2006.
In the year since its adoption many studies have been conducted in accordance with the specified OECD, OPPTS and ISO guidelines. However, it is apparent that these guidelines, which were written primarily with industrial chemicals and agrochemical products in mind, need careful interpretation when used for the evaluation of pharmaceutical products to ensure meaningful data is derived.
A key parameter for predicting the environmental fate of a compound is the adsorption coefficient (Koc) normalised for the organic carbon content of the sorbent, soil or sewage sludge. We have observed unusual binding patterns with some compounds, with the highest levels of binding to the substrates having the lowest organic carbon content.
In the study of fate in aquatic sediments, the selection of the treatment level has to be a balance between the PEC (predicted environmental concentration), water solubility, analytical sensitivity and compound mode of action. For biodegradation, interrogation of an extensive database has shown few compounds can be classified as readily biodegradable, this can be attributed to the test methodology and alternative approaches need to be considered. The fish bioconcentration study has also yielded unexpected data, the highest levels of accumulation sometimes being observed with compounds having lower log Pow values.
In conclusion, the traditional thinking associated with generally lipophilic agrochemicals cannot be applied to medicinal products. An 'intelligent testing' approach is needed, that utilises the specified guidelines in a way that ensures the development of a data set to address the regulatory requirements, whilst taking into account the unique chemical characteristics and routes of entry to the environment of human medicinal products.

2011 COMPUTATIONAL TOXICOLOGY: THE PUBLIC HEALTH PERSPECTIVE.

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ATSDR's Computational Toxicology and Methods Development Laboratory implements a full range of methods in support of the Agency mission to protect human populations from exposure to environmental contaminants. These methods

include benchmark dose (BMD), chemical-specific adjustment factor (CSAF), physiologically-based pharmacokinetic/pharmacodynamics (PBPK/PD), quantitative structure-activity relationship (QSAR), genetic-susceptibility and meta-analysis modeling, and modeling the toxicity of chemical mixtures. Computational toxicology methods are used as an integrated systematic approach in the development of ATSDR Minimal Risk Levels to be used as health guidance values to protect populations exposed to toxic chemicals at hazardous waste sites. For example, this approach has been applied for derivation of an acute oral MRL of 0.5 mg/kg/day for methylene chloride, an intermediate oral MRL of 0.2 mg/kg/day for boron, and a chronic oral MRL of 0.0003 mg/kg/day for methyl mercury. These methods are also used in the development of ATSDR Toxicological Profiles, to support environmental health consultations and prioritization of environmental chemical hazards when experimental information is insufficient, and to improve study design when filling priority data needs as mandated to ATSDR by the U.S. Congress.

2012 DATA-DERIVED UNCERTAINTY FACTOR APPROACH IN THE REVISED N-METHYL CARBAMATE CUMULATIVE RISK ASSESSMENT.

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EPA completed its Revised Cumulative Risk Assessment (CRA) of the N-methyl Carbamate (NMC) Pesticides in 2007. This assessment evaluated the joint risk to 10 pesticides from food, water, and residential exposure. NMCs share the ability to inhibit acetylcholinesterase (AChE) via carbamylation of the active site leading to accumulation of acetylcholine and ultimately clinical signs. Adult rat brain data provided the basis for relative potency factors and points of departure in the NMC CRA. The Agency used a data-derived uncertainty factor approach to evaluate both the interspecies (animal to human) factor and the FQPA 10X factor specific to infants and children. Where there were no human study or comparative sensitivity data for individual NMCs, respective factors remained 10X. Data from toxicity studies with human subjects were used to quantify the interspecies factor for three pesticides (aldicarb, methomyl, oxamyl). These studies were evaluated by the Human Studies Review Board in 2006 and considered appropriate for purposes of risk assessment. A sensitivity analysis was performed where a 3X factor for interspecies extrapolation was applied to the other NMCs without data in human subjects. This 3X factor was used instead of the typical 10X factor based on the assumption of pharmacodynamic equivalence for interspecies extrapolation. Data collected in comparative AChE studies where adult and post-natal day 11 or 17 pups were exposed via gavage were used to inform the magnitude of the FQPA 10X factor for infants and children. In order to provide a uniform measure of comparison for these factors, a benchmark dose approach was used. The BMD10 or the dose expected to cause 10% inhibition in AChE was estimated for adult rat, adult human, and juvenile rat AChE data. This data-derived approach to applying uncertainty and extrapolation factors represents a major advance in improving the scientific basis for risk extrapolation with pesticide chemicals.

2013 POLYCYCLIC AROMATIC HYDROCARBON (PAH) MIXTURES: ASSESSMENT OF CANCER RISK USING A PROPOSED RELATIVE POTENCY FACTOR (RPF) APPROACH.

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U.S. EPA's Integrated Risk Information System (IRIS) Program is undertaking a health assessment for PAH mixtures that considers a RPF approach which provides a cancer risk estimate by summing the estimated potency of individual PAHs relative to an index compound (benzo[a]pyrene, BaP). The current effort focuses on available data from: 1) cancer bioassays and cancer-related endpoints (e.g., mutagenicity) for individual PAHs in which at least one PAH was studied in addition to BaP; and 2) mode of action studies and studies related to additivity of effects. A framework has been proposed for ranking studies based on study quality and relevance to risk for human cancer, and a weight-of-evidence approach has been developed to inform the derivation of RPFs. Draft RPFs were calculated by comparing potency estimates derived from benchmark dose modeling when possible and a point estimate approach was used when modeling was not feasible. Selected RPF values were given a relative confidence rating based on the quality and consistency of the overall database, as well as the quality of the principal study(s) on which the RPF is based. Data arrays have been developed to illustrate the variability of the relative potency measures in the database for each individual PAH. Some of the areas of uncertainty in the approach include: (1) contributions to carcinogenicity from PAHs without adequate data for developing RPFs; (2) potential non-additive interactions among components of whole PAH mixtures; and (3) extrapolation of human cancer risks from animal studies and in vitro data. (The views expressed are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA).

2014 MODE OF ACTION FRAMEWORKS FOR RISK ASSESSMENT: CHALLENGES AND OPPORTUNITIES FOR PROGRESS.

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Mode of action (MOA) information is increasingly being applied in human health risk assessment. The MOA can inform issues such as the relevance of observed effects in laboratory animals and variability of response within the human population. A framework for analyzing MOA information in human health risk assessment of environmental carcinogens has been adopted by the WHO-International Programme on Chemical Safety, International Life Sciences Institute and US Environmental Protection Agency. Overall the framework approach, which has been expanded to early life and developmental effects, has enhanced the incorporation of biological data in environmental chemical health risk assessment. We identify herein challenges and opportunities for further progress. One challenge is to articulate the significant role of scientific judgment. A related issue is to consider alternatives to binary (yes/no) questions that can falsely dichotomize options, discourage scientific consensus for complex decisions, and inappropriately narrow consideration of data that can nonetheless inform the overall assessment of risk or attendant uncertainties. Future effort may benefit from a broad consideration of the known causes of human disease and the potential for chemical effects to act additively with these as well as endogenous background processes. Another area for improvement is the evaluation of multiple MOAs over the same dose range, or of varying MOAs at different life stages. Instead of considering each MOA and outcome individually, analyses may be able to address how multiple metabolites, modes, and toxicity pathways contribute to the overall toxicological profile. This would facilitate the use of toxicogenomic data covering multiple genes, molecular targets, and toxicity pathways. An additional opportunity for progress is the extension of the MOA analysis across outcomes known to have common modes. Finally, the attendant quantitative implications also deserve further consideration. Disclaimer: These views are those of the authors and do not necessarily reflect US EPA policy or opinion.

2015 MUTAGENIC MODE OF ACTION FOR CARCINOGENICITY: COMMENTS ON EPA'S DRAFT FRAMEWORK.

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In U.S. EPA's 2005 revised Guidelines for Carcinogen Risk Assessment, determination of a mode of action (MOA) for an agent is the key decision in hazard identification and dose response. In tandem with the Cancer Guidelines, EPA published guidance on assessing special risks of early life exposure to carcinogens; this guidance includes numerical adjustments to cancer potency, for putative carcinogens judged to have a mutagenic MOA. To expand and clarify discussions on issues evaluated for a mutagenic MOA for carcinogenicity, EPA has released for public comment the draft Framework for Determining a Mutagenic Mode of Action for Carcinogenicity: Using EPA's 2005 Cancer Guidelines and Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens. The framework includes these steps: 1. organization of data; 2. assessment of data quality; 3. determining the weight of evidence that the chemical is mutagenic (vs. other types of genotoxic activity); 4. application of EPA's MOA framework (from the Cancer Guidelines) to determine if a chemical has a mutagenic MOA. The document includes discussion of utility of specific genotoxicity assays to determine a mutagenic MOA. The opinions in this abstract are those of the authors and not necessarily policy of their Agencies.

2016 INTEGRATION OF TOXICOGENOMICS DATA INTO MODE OF ACTION ANALYSES FOR CANCER RISK ASSESSMENT.

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An understanding of the role of genotoxicity in carcinogenesis is considered an essential element in the characterization of mode of action (MOA) for carcinogenicity. The determination of a mutagenic MOA informs the low-dose extrapolation

used for risk estimation of potential carcinogens currently under evaluation in U.S. EPA's Integrated Risk Information System (IRIS) Program. Standard genotoxicity testing is generally considered to provide accurate hazard detection; however, interpretation of positive findings in terms of relevant risk to humans is difficult because of limited insight into the underlying mechanisms. The development of new experimental approaches capable of differentiating a wide range of genotoxic mechanisms is expected to improve carcinogenic characterization EPA's 2005 Guidelines for Carcinogen Risk Assessment encourage the incorporation of data from new technologies to better inform risk assessment. Toxicogenomic (TG) data are beginning to be used in risk assessment to inform MOA analyses and support weight-of-evidence evaluations for the characterization of carcinogenicity of environmental chemicals. One of the hallmarks of TG is identification of molecular signatures or distinctive fingerprints of specific classes of toxic compounds that discriminate direct-acting from indirect-acting genotoxicants based on their gene expression patterns. The goal of this presentation is to summarize current scientific progress in TG methodologies and to integrate such data for evaluating and characterizing genotoxic from non-genotoxic carcinogens. The information obtained from such analyses can be utilized to improve understanding of the MOA for environmental carcinogens and to facilitate better cancer risk characterization. (The views expressed are those of the authors and do not necessarily reflect the views or policies of the U.S EPA).

2017 IS VALPROIC ACID TERATOGENICITY DEPENDENT ON HISTONE DEACETYLASE INHIBITION?

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The teratogenic effects of valproic acid (VPA), a potent inducer of neural tube defects (NTD), have been proposed to be mediated by histone deacetylase (HDAC) inhibition. Our previous microarray studies in P19 mouse embryocarcinoma cells and mouse embryos revealed that VPA may deregulate the expression of a large number of genes, many of which have critical roles in neural tube formation and closure. In this study we utilize VPA analogs as tools to define gene expression responses related to teratogenicity and HDAC inhibition. We exposed undifferentiated R1 mouse embryonic stem (ES) cells to 1 mM VPA, 0.25 mM or 0.5 mM S-2-pentyl-4-pentynoic acid (more potent teratogen and HDAC inhibitor than VPA), 1 mM 3-propyl-heptanoic acid (potent teratogen but not an HDAC inhibitor), 1 mM 2-ethyl-4-methyl-pentanoic acid (potentially pharmacologically active non-teratogen and non-HDAC inhibitor), or vehicle (0.1% DMSO) for 6 h, after which RNA samples (n=3) were subjected to microarray analysis using CodeLinkTM Mouse Whole Genome Bioarrays. We found VPA to deregulate a large number of genes in the ES cells. Similarly large numbers of genes were deregulated by the teratogenic VPA analogs, including the non-HDAC inhibitor, but only a few by the non-teratogen. Excluding genes with a response in the non-teratogenic treatment, we found 102 genes in common (>2-fold change and adjusted p value<0.001) between the partly overlapping yet distinct sets of genes deregulated in the teratogenic treatments. Known NTD-related genes were overrepresented in the teratogenic treatments, independently of HDAC inhibition, and had higher average fold changes. These results indicate that the teratogenicity of VPA may be linked to its ability to deregulate the expression of a large number of genes, many of which may be deregulated by other mechanisms than HDAC inhibition.

2018 DEVELOPMENTAL TOXICITY OF PERFLUOROOCTANE SULFONATE (PFOS) IS NOT DEPENDENT ON EXPRESSION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-ALPHA (PPARα) IN THE MOUSE.

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Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are members of a family of perfluorinated compounds and both are environmentally persistent and found in the serum of wildlife and humans. PFOS and PFOA are developmentally toxic in rats and mice. Exposure in utero reduces postnatal survival and growth and delays development. Exposure to PFOS on the last 4 days of gestation in the rat is sufficient to reduce neonatal survival. PFOS and PFOA are weak agonists of PPARα and exposure results in liver enlargement. The reduced postnatal survival of neonatal mice exposed to PFOA was recently shown to depend on expression of PPARα. This study used PPARα knockout (KO) and 129S1 wild type (WT) mice (Jackson Laboratories, Bar Harbor, ME) to determine if PPARα expression is required for the developmental toxicity of PFOS. After mating overnight, the next day was designated gestation day (GD) 0. WT females were weighed and dosed orally from GD15-18 with water, 4.5, 6.5, 8.5, or 10.5 mg PFOS/kg/day. KO females were dosed with water, 8.5 or 10.5 mg/kg/day. Dams and pups were observed daily and pups were weighed on postnatal day (PND) 1 and PND15, eye opening was recorded from PND12-15. Dams and pups were killed on PND15, body and liver weights recorded, and serum collected. PFOS did not affect GD15-18 weight gain, or PND15 body or liver weights of the dams. Neonatal survival (PND1-15) was significantly reduced by PFOS in both WT and KO litters at all doses. Pup weight gain from PND1-15 was not significantly affected by PFOS exposure in either group. Relative liver weight of WT and KO pups was significantly increased by the 10.5 mg dose. Eye opening of PFOS-exposed pups was slightly delayed in WT and KO on PND13 or 14, respectively, $p<0.05$ versus controls. Because results in WT and KO were comparable, it is concluded that PFOS-induced neonatal lethality and delayed eye opening are not dependent on activation of PPARα. This abstract does not necessarily reflect US EPA policy.

2019 HUMAN FETAL LIVER HEMATOPOIETIC STEM CELLS AS A TOOL FOR UNDERSTANDING *IN UTERO* INJURY OF TRANSPLACENTAL CHEMICALS.

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During development, human fetal liver hematopoietic stem cells (HSC) comprise a significant percentage of hepatic cell populations and are the primary progenitor cells that confer hematopoiesis. Because the fetal liver receives a substantial proportion of maternal blood flow during pregnancy, these cells can be fetal targets of maternal chemicals. Furthermore, the critical function of fetal liver HSC in the development of hematopoiesis suggests that injury to these cells can have ramifications with regards to the etiology of hematopoietic disorders that can develop after birth. Our laboratory has developed a primary culture system for human derived fetal liver HSC and has been using this model to examine the mechanisms of toxicity of a number of transplacental agents, including pesticides, polybrominated diphenyl ethers (PBDEs), oxidative damage products, and anticancer drugs. We have observed that fetal liver HSC are sensitive to the acute toxicity of a number of these compounds, and our studies suggest that that the sensitivity may be due, in part, to poor detoxification and DNA repair capabilities. Exposure to etoposide, an anticancer drug that can produce secondary leukemias in adults following chemotherapy, induces the formation of mixed lineage leukemia (MLL) gene rearrangements in fetal liver HSC. These findings are significant as MLL gene rearrangements are the signature genetic lesions found in leukemic blasts of infants with leukemia and are formed in utero prior to disease onset. Other studies in our laboratory indicate that mitochondrial injury and oxidative damage are mechanisms of BDE 47 fetal HSC injury. Collectively, our studies demonstrate the utility of using primary cultures of human fetal liver HSC to investigate mechanisms of toxicity of relevance in utero, and also for studies addressing the uncertainties associated with chemical exposures during pregnancy and prenatal injury. Supported by NIH ES-007032, NIH-HD00836, NOAA NA05NOS4781253.

2020 PREFERENTIAL EXPRESSION OF XENOBIOTIC EFFLUX TRANSPORTERS IN MOUSE FETAL MEMBRANES COMPARED TO PLACENTA.

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Fetal exposure to xenobiotics and toxicants can be restricted by transporters at the interface between maternal and fetal circulation. Previous work has identified a number of transporters in the placenta, however, less is known about the presence of these transporters in yolk sac and amnion membranes, together known as the fetal membranes. The purpose of this study was to quantify mRNA and protein expression of xenobiotic transporters in C57BL/6 mouse placenta and fetal membranes during mid- to late-gestation. Concepti (day 11) or placenta and fetal membranes (days 14 and 17) were collected from pregnant mice and analyzed for multidrug resistance-associated proteins (Mrps), multidrug resistance protein (Mdr), breast cancer resistance protein (Bcrp) and organic anion transporting polypeptides (Oatps) mRNA (bDNA assay) and protein (western blot and immunofluorescence). Mrp 2-7, Mdr1b, Bcrp, Oatp2a1 and 5a1 mRNA were detected in day 11 concepti. By days 14 and 17, fetal membranes expressed higher levels of the efflux transporters Mrp2 (7-fold), Mrp4 (4-fold), Mrp5 (3-fold), Mrp6 (11-fold) and Bcrp (2-fold) mRNA compared to placenta. Western blot analysis of Mrp2, Mrp4 and Bcrp revealed increased expression of these efflux proteins in fetal membranes compared to placenta. Mrp5 protein was expressed at a similar level in fetal membranes and placenta. Immunostaining revealed apical (Mrp2, Bcrp) and basolateral (Mrp4, 5, 6) cellular localization in epithelial cells of the yolk sac. In contrast, the uptake carriers Oatp2a1 and 5a1 had placenta-predominant mRNA

expression. The mRNA levels of Mrp3, Mrp7 and Mdr1b were similar between placenta and fetal membranes during late gestation. In conclusion, xenobiotic transporters in the fetal membranes provide an additional route to protect the fetus against endogenous chemicals as well as environmental contaminants and other xenobiotics. (Supported by NIH ES-09649, ES-09716, ES-07079, ES-013714, RR021940)

2021 PARACRINE HH SIGNALING IN PROSTATE: STAGE-SPECIFIC EFFECTS ON EPITHELIAL PROLIFERATION.

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Hedgehog (Hh) signaling plays an important role in prostate development. Recent studies on Hh signaling in prostate development and prostate cancer suggest that paracrine (epithelial to mesenchymal) Hh signaling exerts a variety effects in epithelial proliferation and differentiation. Here, we used both *in vivo* and *in vitro* methods to determine the paracrine Hh effect on epithelial proliferation during prostate development. An immortalized mesenchymal cell line-UGSM-2 was transfected with activated form of Hh signaling intermediates Smo/Gli2 to simulate an activated ligand independent Hh paracrine pathway. UGS tissues isolated from both prenatal and postnatal stages were co-cultured with UGSM-2 cells in explant culture plates for 1 week. Tissues were BrdU labeled 2 hours prior to fixation in 10% formalin. Paraffin embedding and immunostaining was performed for BrdU, and Pan-cytokeratin. The results showed that the paracrine Hh signals promote epithelial budding and proliferation at the stage of bud initiation (prenatal), while inhibit epithelial proliferation on the initiated buds (postnatal). We also generated a mouse model that has activated paracrine Hh signaling by cre-lox mating system. IHC staining on prostates from both prenatal and postnatal stage showed consistent results with *in vitro* explants co-culture experiment data. This suggests stage-specific Hh responsiveness during normal prostate development, which introduces a novel paradigm into our efforts to understand normal growth regulation and offers important insights into the role of Hh signaling in prostate cancer.

2022 ARYL HYDROCARBON RECEPTOR REGULATES WILM'S TUMOR SUPPRESSOR GENE FUNCTION DURING NEPHROGENESIS.

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The molecular mechanisms governing ontogenesis are regulated by somatic and environmental factors in utero that activate or repress the expression of numerous genetic elements. The aryl hydrocarbon receptor (AHR) is an important ligand-activated nuclear transcription factor involved in developmental programming. Recent studies in this laboratory have shown that modulation of AHR signaling by genetic ablation, mutation or exogenous environmental ligands induces deleterious developmental deficits in the kidney. Benzo(a)pyrene (BaP), acting as an AHR ligand, inhibits nephrogenesis in embryonic day 11.5 C57/bL/6J (C57) mouse embryos and alters -KTS/+KTS Wt-1 mRNA ratios. These findings are significant since Wilms' Tumor suppressor gene (WT-1) encodes a Cis_2-His_2 zinc finger protein that mediates transcriptional repression/activation of genes during mesenchymal-to-epithelial (MET) trans-differentiation. To further evaluate functional interactions between AHR and WT-1 during nephrogenesis, studies were conducted to examine the molecular consequences of disruption in AHR signaling by small interfering RNA or repeated exposures to BaP (3 μM) or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (1 nM) in metanephric kidney cells (mK4). The loss of AHR protein induced by these diverse manipulations consistently altered WT-1 mRNA ratios and inhibited expression of the Wt-1 transcriptional targets Syndecan 1, Pax2, Egf- receptor, and RaRα. These changes correlated with marked downregulation of renal differentiation markers, including Sfrp1, Igf1r, Igf2r, Wnt4, Lhx1, and E-cadherin. Over-expression of Wt-1 splice variants in mK4 cells differentially down-regulated WT-1 targets as a function of KTS or exon 5 inclusion/exclusion. Thus, AHR signaling may regulate nephrogenesis in vivo by coupling changes in Wt-1 mRNA splice variant ratios with functional deficits in downstream WT-1 targets. On the basis of these findings we propose a model for examining the impact of genetic variance in AHR signaling on nephrogenic outcomes.

2023 INVOLVEMENT OF INFLAMMATION-RELATED GENES IN TCDD-INDUCED HYDRONEPHROSIS IN MALE C57BL/6 MOUSE PUPS.

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Hydronephrosis is caused by *in utero* and lactational exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), but the mechanism underlying its pathogenesis is unknown. Previous studies using knockout mice have demonstrated that AhR is essential for the onset of TCDD-induced hydronephrosis [Mimura et al., 1997], but that induction of CYP1A1, CYP1A2 and CYP1B1 are not required [Dragin et al., 2006]. Here, we sought to identify novel genes involved in TCDD-induced hydronephrosis. Female dams were orally administered TCDD (10 μg/kg) the first day after delivery. Male pups exposed to TCDD via lactation were examined for alterations in kidney gene expression on postnatal days (PNDs) 3, 5, 7, 9 and 11. At PND 9, cyclooxygenase-2 (COX-2) was significantly up-regulated and urinary amounts of prostaglandin E_2 (PGE_2) were increased. Furthermore, inhibition of COX-2, by a selective COX-2 inhibitor, abolished both the increase in urinary PGE_2 excretion and the development of hydronephrosis. Quantitative real-time RT-PCR analyses of the mRNA abundance of inflammation-related genes in the kidneys of TCDD-exposed male mouse pups showed sequential up-regulation of MCP-1, followed by TNF-α and IL-1β and F4/80, and then later by COX-2. To clarify the role of COX-2 in TCDD-induced hydronephrosis, COX-2 mRNA abundance in the male pup kidney on PND9 was up-regulated by daily administration of lithium chloride to the dams from PND1 to PND21, but hydronephrosis failed to develop. Thus, the sequential up-regulation of these inflammation-related genes may underlie the development of hydronephrosis caused by TCDD, and COX-2 may be an essential, but not sufficient factor, for TCDD-induced hydronephrosis.

2024 EFFECTS OF 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN ON BONE MINERALIZATION IN THE GROWING MOUSE.

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Exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induces a characteristic toxic lesion in the distal tubules which are known to be the main sites for Ca reabsorption and for synthesis of the active form vitamin D. In order to investigate whether TCDD disturbs Ca homeostasis and vitamin D metabolism, effects of TCDD on the expression of genes involved in vitamin D metabolism and Ca2+ reabsorption were analyzed in the developing mouse kidney. Mouse pups were exposed to TCDD lactationally, and sacrificed on postnatal day (PND) 7, 14, and 21 for biochemical analysis. Real time RT-PCR was performed on total RNA derived from the kidney. Tibia from TCDD-exposed mouse pups were also examined histomorphometrically, TCDD up-regulated markedly both CYP 27b1-protein and mRNA, enzyme to catalyze synthesis of the active metabolite of vitamin D as well as a significant increase in serum vitamin D levels, but no changes in serum Ca levels. While TCDD down-regulated both calbindin D and NCX-1 (Na+/Ca2+ exchanger) mRNA expressions which are involved in the transcellular Ca2+ transport in the kidney, both mRNAs were up-regulated in intestine in early postnatal period. Not only serum osteocalcin levels, but also both osteocalcin and alkaline phosphatase mRNAs in tibia were significantly down-regulated by TCDD. Histomorphometric analysis of tibia from TCDD-treated mouse revealed abnormal bone histology identical with that of renal osteodystrophy characterized by a gain in volume and thickness of osteoids accompanied with an abnormality of mineralization in the cortical bone, suggesting that the inhibitory effects on bone formation may be responsible for a TCDD-induced bone toxicity.

2025 ADVERSE HEATH OUTCOMES IN ADULT OFFSPRING OF RATS UNDERNOURISHED *IN UTERO* – STRAIN COMPARISON FOR USE IN DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE (DOHAD) TOXICITY STUDIES.

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The DOHaD hypothesis proposes that environmental factors in early life alter the susceptibility to adult disease. Gestational undernutrition in humans can result in birth weight deficits and, when postnatal nutrition is dissimilar, predisposition to diseases in adulthood including: insulin resistance, glucose intolerance, high blood pressure and obesity. Comparable studies have been conducted in rats, yielding variable results depending on the strain and deprivation period. This study compares adverse outcomes following 50% global undernutrition on gestation days (GD) 1-15 (UN1-15) or GD 10-21 (UN10-21), in Sprague Dawley (SD) and Wistar (W) rats, to control rats fed a chow diet ad libitum throughout gestation. Undernourished pups were cross-fostered to control dams at birth. SD and W UN10-21 pups, but not UN1-15 pups, had a significantly lower birth weight. Catch-up growth occurred in the UN10-21 pups by postnatal day 14. At weaning, male pups were retained and fed either a normal (N) or a high fat (HF) diet. Oral glucose tolerance testing was conducted at 10 weeks of age. Insulin responses were

similar regardless of prenatal nutrition in both strains. Post-weaning HF diet feeding heightened the insulin response compared to N diet-fed rats; statistically significant in the SD strain. Tail-cuff systolic blood pressure (SBP) was measured at 11 weeks of age. In SD rats, SBP was elevated in both in utero undernutrition groups, compared to control groups. The post-weaning diet had no additional effect on SBP. In contrast, W rats had similar SBP regardless of prenatal nutrition or post-weaning diet. These results suggest that SD rats are more sensitive than W rats to adverse adult health outcomes following a suboptimal in utero environment. This study provides critical comparative data for utilization in future DOHaD studies. This abstract does not necessarily reflect EPA policy.

2026 REGULATORY MECHANISM OF NRF2 ACTIVATION BY HYPOCHLOROUS ACID AND CONCOMITANT ACTIVATION OF INFLAMMATORY PATHWAYS.

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Chlorine is an important commercial chemical with substantial industrial and domestic use. When chlorine reacts with water in the respiratory airways, a potent oxidant, hypochlorous acid (HOCl) is generated. Transcription factor NF-E2-related factor 2 (Nrf2)-mediated antioxidant response represents a critical cellular defense mechanism that serves to maintain intracellular redox homeostasis and limit oxidative damage. We recently demonstrated that HOCl activates Nrf2 in cultured mouse macrophages, one of the target cells of chlorine exposure. In the current study we found that HOCl-induced Nrf2 activation showed a two phase dose-dependent response in RAW 264.7 macrophages. In 6 hrs low concentrations of HOCl (up to 1.4 mM) caused a significant increase in Nrf2 nuclear accumulation, DNA binding, expression of Nrf2 target genes as well as intracellular levels of glutathione. In contrast to these responses observed at low doses, when the HOCl concentration reached 2.8 mM, which has no obvious cytotoxic effect in 6 hrs, the induction of Nrf2 target genes and GSH levels was dramatically reduced, whereas the expression of several inflammatory cytokines including IL6 and IL1β significantly increased. In addition, activation of inflammatory pathways with LPS or TNFα led to an attenuation of HOCl-induced Nrf2 activation. To identify key regulators of HOCl-induced Nrf2 activation, we used a sensitive antioxidant response element (ARE)-reporter cell line (AREc32) to screen an siRNA library with targets for over 2000 kinases and phosphatases along with a wide range of biologically active kinase inhibitors. Among the kinases that were required for Nrf2 activation by HOCl included CK2, PI3K and p38. Taken together, these findings suggest a critical role of protein phosphorylation in Nrf2-mediated antioxidant defense to HOCl exposure and a potential interaction of Nrf2-mediated antioxidant response and cellular inflammatory response caused by HOCl.

2027 COMPARISON OF CITRUS COUMARINS ON CARCINOGEN-DETOXIFYING ENZYMES IN NRF2(-/-) VS WILD-TYPE MICE.

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Citrus coumarins possess anti-carcinogenic activities in several animal models. Imperatorin, isopimpinellin, and auraptene blocked 7,12-dimethylbenz[a]anthracene DNA adduct formation in mouse mammary glands. These coumarins also induced phase II carcinogen-detoxifying enzymes such as glutathione S-transferase (GST) and NAD(P)H quinone oxidoreductase (NQO1). Nrf2 is a positive regulator of the antioxidant response element (ARE), which is a mechanism for induction of phase II enzymes. To determine whether citrus coumarins induce GST via these mechanisms, two approaches were used. First, HepG2 cells stably transfected with the ARE and a green fluorescent protein (GFP) reporter were treated with increasing concentrations (6.25-100μM) of imperatorin, isopimpinellin, auraptene, and positive controls tert-butylhyqroquinone (TBHQ) and oltipraz. TBHQ and oltipraz increased GFP fluorescence by 280% and 185%, respectively compared to the control. Imperatorin and auraptene increased GFP fluorescence by 144% and 121%, respectively. Isopimpinellin did not increase GFP fluorescence. Next, the effects of orally-administered imperatorin, isopimpinellin, auraptene, and the positive control oltipraz on hepatic GST activities were compared in Nrf2 (-/-) knockout and wild-type (WT) mice. Oltipraz, imperatorin, isopimpinellin, and auraptene all significantly increased GST activity in WT mice using 1, 2-dichloro-3-nitrobenzene (DCNB) as a substrate. In contrast, GST activity was not significantly increased by oltipraz, imperatorin, or auraptene in the Nrf2 (-/-) knockout mice. Isopimpinellin partially increased GST activities in the Nrf2 (-/-) knockout mice. These results strongly suggest that imperatorin and auraptene induce GST via the Nrf2/ARE mechanism. Although structurally similar, isopimpinellin appears to induce GST via additional mechanisms. 1K22 CACA102005-01A2

2028 TARGETED DISRUPTION OF THE NUCLEAR FACTOR-E2-RELATED FACTOR-2 *(NRF2)* GENE PREVENTS FORMATION OF CHOLESTEROL GALLSTONES IN MICE.

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Nuclear factor-E2-related factor-2 (Nrf2) determines the basal and inducible expression of many detoxifying enzymes and some transporters in liver. The aim of this study was to determine whether Nrf2 influences cholesterol gallstone formation. Nrf2-null and wild-type mice of both genders were fed a lithogenic diet containing 1% cholesterol, 0.5% cholic acid and 15% butterfat for 8 weeks, the common bile duct was cannulated for measurement of biliary lipid secretion rates. Gallstones were quantified by microscopy and protein expression was analyzed by Western blot. After 8 weeks of consuming the lithogenic diet, all wild-type mice formed cholesterol monohydrate crystals and/or gallstones. In contrast, Nrf2-null mice did not have any detectable cholesterol crystals or gallstones in gallbladder bile. Compared with Nrf2-null mice, wild-type mice displayed significantly larger gallbladders, a higher bile flow rate, and greater biliary lipid secretion. On the chow diet, expression levels of basolateral efflux transporters multidrug resistance-associated protein (Mrp) 1 and 3-6 were basically similar between two groups of mice. Bile salt-export pump (Bsep) expression was lower and breast cancer resistance protein (Bcrp) expression was higher in livers of the gallstone susceptible wild-type mice as compared to that in gallstone sensitive Nrf2-null mice. Feeding the lithogenic diet significantly increased expression levels of Bcrp and Mrp3 in wild-type mice, but to a lesser extent in Nrf2-null mice. Moreover, after feeding the lithogenic diet, expression levels of Mrp2 were significantly increased in Nrf2-null mice. In summary, deletion of the Nrf2 gene prevents cholesterol gallstone formation in mice. Its protective actions may be associated with hepatic lipid transporters and the mechanism is being intensively investigated.

2029 HEPATOCYTE-SPECIFIC DELETION OF *KEAP1* ALTERS EXPRESSION OF HEPATIC DRUG METABOLIZING ENZYMES AND TRANSPORTERS.

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Hepatocyte-specific deletion of *Keap1* results in constitutive activation of the nuclear factor-E2-related factor-2 (Nrf2). In addition to regulating detoxification and antioxidant gene expression, Nrf2 also modulates expression of some efflux transporters such as Multidrug resistance-associated proteins (Mrp) 2, 3, and 4. Therefore, constitutive Nrf2 activation may influence expression of hepatic transporters in mice with hepatocyte-specific deletion of *Keap1* which may contribute to altered chemical disposition in liver and increased resistance to drug/chemical-induced hepatotoxicity. In the current study we determined the mRNA and protein expression of several drug metabolizing enzymes and transporters in livers of wild-type and hepatocyte-specific *Keap1*-null mice. Several cytoprotective enyzmes showed increased mRNA and/or protein expression in the livers of *Keap1*-null mice, including sulfotransferase 2a1/2, NADP(H):quinone oxidoreductase 1, cytochrome P450 2b10, 3a11, and glutamate-cysteine ligase (catalytic subunit). In regard to hepatic uptake, *Keap1*-null mice had higher mRNA and protein expression of Organic anion transporting polypeptide (Oatp) 1b2 in liver, but almost complete abolishment of Oatp1a1, compared to wild-type. By contrast, efflux transporters Mrp1-5 showed higher expression at both mRNA and protein levels in livers of *Keap1*-null mice, with particularly Mrp4 more than 15-fold higher than wild-type. Taken together, these data indicate that constitutive Nrf2 activation can alter hepatic uptake, metabolism and efflux, and thus Nrf2 may play a large role in hepatic protection and cellular resistance to toxic chemicals. (This work was supported by RI-INBRE grant #P20RR016457 from NCCR, NIH)

2030 THE NRF2 ACTIVATOR OLTIPRAZ ALSO ACTIVATES THE CONSTITUTIVE ANDROSTANE RECEPTOR.

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Oltipraz (OPZ) is a well-known inducer of NAD(P)H:quinone oxidoreductase (NQO1) along with other enzymes that comprise the NF-E2-related factor2 (Nrf2) battery of detoxification genes. However, OPZ treatment also induces expression of CYP2B, a gene regulated by the constitutive androstane receptor (CAR). Therefore, this study was designed to determine whether OPZ induces gene expression in the mouse liver through activation of CAR in addition to Nrf2. OPZ increased the mRNA expression of both CYP2B1/2 and NQO1 in C57BL/6 mouse livers. As expected, in livers from Nrf2-/- mice, OPZ induction of NQO1 was reduced, indicating NQO1 induction is dependent on Nrf2 activation. CYP2B10 induction by OPZ was unchanged. The robust induction of CYP2B by OPZ in wild-type mice was completely absent in CAR-/- mice, revealing a CAR dependent induction by OPZ. OPZ also induced transcription of the human CYP2B6 promoter-reporter containing the phenobarbital responsive element in mouse liver using an in vivo transcription assay. Additionally, OPZ induced in vivo nuclear accumulation of CAR at 3 h, but, as with phenobarbital, was unable to reverse androstanol repression of mCAR constitutive activity in transiently transfected HepG2 cells. In summary, OPZ induces expression of CYP2B and NQO1 via the activation of CAR and Nrf2, respectively.

2031 ACETYLTRANSFERASE P300/CBP REGULATES NRF2-DEPENDENT ANTIOXIDANT RESPONSE THROUGH ACETYLATION-DEPENDENT AND INDEPENDENT MECHANISM.

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Nrf2 is a critical transcription factor that regulates endogenous antioxidants, phase II detoxification enzymes and other cellular defensive proteins through the antioxidant responsive element (ARE) in the promoter regions of these genes. Understanding the molecular mechanisms by which Nrf2 is regulated is important for pharmaceutical intervention of the antioxidant signaling pathway. We have previously shown that Keap1, functioning as a molecular switch, targets Nrf2 for ubiquitin-mediated proteasomal degradation under normal cellular redox conditions. Upon oxidative stress, the enzymatic activity of Keap1-dependent ligase is inhibited, leading to Nrf2 protein accumulation and enhanced Nrf2-dependent transactivation. In addition to keap1-dependent on-and-off regulatory mechanism in controlling Nrf2 activity, here we further show additional levels of regulation within the nucleus. Histone acetyltransferase p300/CBP (CREB binding protein) fine-tunes Nrf2 functions through both acetylation-dependent and independent mechanisms. We have characterized the physical and functional interactions between p300/CBP and Nrf2, and have identified specific lysine residues within Nrf2 that is acetylated by p300/CBP. These findings clearly demonstrate the complexity of Nrf2 regulation, which provides insights into how Nrf2 signaling is integrated with other signaling pathways through interacting with transcription co-activators within the nucleus.
(NIEHS 1RO1ES015010-01)

2032 NUCLEAR FACTOR ERYTHROID 2-RELATED FACTOR 2 (NRF2) INHIBITS CYTOKINE PRODUCTION BY ACTIVATED MURINE T CELLS.

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Nrf2 is a transcription factor that is activated by oxidative stress and electrophilic molecules. Nrf2 is best known for its role in the induction of antioxidative genes and other detoxification mechanisms. However, recent studies have demonstrated that Nrf2 also plays an anti-inflammatory role in a variety of models. The majority of these studies have focused upon direct effects of Nrf2 in macrophages and other myeloid cells. The present studies demonstrate that Nrf2 also regulates cytokine production in T cells. The Nrf2 activators, *tert*-butylhydroquinone (tBHQ) and butylated hydroxyanisole (BHA), suppress IFNγ secretion by anti-CD3/anti-CD28-activated splenic T cells in a concentration-dependent manner. Likewise, tBHQ and BHA suppress IFNγ mRNA levels, demonstrating that the inhibition of IFNγ occurs at the transcriptional level. The inhibition of IFNγ transcription by tBHQ and BHA is observed in wild-type, but not Nrf2-null T cells, indicating the effect is dependent upon Nrf2 and not a non-specific effect. Interestingly, our current studies also show that IFNγ production is markedly greater in activated Nrf2-null T cells compared to activated wild-type T cells, suggesting that Nrf2 plays a role in the regulation of IFNγ in the absence of exogenous Nrf2 activators. In addition, Nrf2 activation abrogates the production of other T cell cytokines, including IL-2 and TNFα. Collectively, these data suggest that Nrf2, in the presence of exogenous activators, plays a regulatory role in activated T cells that ultimately results in the suppression of IFNγ, IL-2, and TNFα production. Furthermore, the present studies demonstrate that inhibition of IFNγ by Nrf2 activators occurs transcriptionally and is Nrf2-dependent. In addition, the aforementioned data suggest that Nrf2 also regulates IFNγ expression in the absence of exogenous activators. (Supported by NIH grants ES09649, ES09716, ES07079, and RR021940.)

2033 IDENTIFICATION OF NRF2-DEPENDENT AIRWAY EPITHELIAL ADAPTIVE RESPONSE TO PROINFLAMMATORY OXIDANT-HYPOCHLOROUS ACID (HOCL) CHALLENGE BY TRANSCRIPTION PROFILING.

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In the airway of inflammatory diseases, high level of HOCl (estimated to be as high as 8mM) can be generated through a reaction catalyzed by leukocyte granule enzyme- Myeloperoxidase (MPO). HOCl, a potent oxidative agent, causes extensive tissue injury through its reaction with various cellular substances including thiols, nucleotides and amines. Besides its physiological source, HOCl can also be generated by chlorine gas inhalation resulting from either an accident or potential terrorist attack. Despite the important role of HOCl induced airway epithelial injury, underlying molecular mechanism is largely unknown. In this study, we found that HOCl induced dose-dependent toxicity in airway epithelial cells. By transcription profiling using Genechip, a battery of HOCl inducible antioxidant genes was identified. And all of them have been reported previously to be regulated by nuclear factor erythroid-2 related factor 2 (Nrf2), a transcription factor that is critical to lung antioxidant response. In consistent to this finding, Nrf2 was found to be activated both time- and dose- dependent of HOCl treatment. Interestingly, although EGFR-MAPK pathway was also highly activated by HOCl, it was not involved in Nrf2 activation and Nrf2-dpendent gene expression. Instead, HOCl induced cellular oxidative stress appeared to directly lead to Nrf2 activation. To further understand the functional significance of Nrf2 activation, small interference RNA (siRNA) was used to either knock down Nrf2 level by targeting Nrf2 or enhance the nuclear accumulation of Nrf2 by targeting its endogenous inhibitor-Keap1. By both methods, we conclude that Nrf2 directly protects airway epithelial cell from HCOL-induced toxicity.

2034 MUTATION OF THE PARKINSON'S GENE DJ-1 INCREASES THE INFLAMMATORY RESPONSE IN MOUSE ASTROCYTES.

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Mutations in the DJ-1 gene have been associated with development of early onset autosomal recessive Parkinson's disease (PD), however, the precise mechanism leading to parkinsonism remains largely elusive. Using gene trap technology, a screening library was created in embryonic stem cells, the DJ-1 gene was mutated and chimeric mice were generated for the in vivo study of cellular pathways controlled by the DJ-1 gene. Real-time PCR affirmed DJ-1 RNA expression was virtually eliminated in DJ-1-/- (KO) mice compared to DJ-1+/+ (WT) controls. Western blot analysis also revealed no DJ-1 protein was synthesized in DJ-1-/- brain tissue. Primary cortical astrocytes from WT and KO animals were treated with the pro-inflammatory stimulant lipopolysaccharides (LPS), and RNA or protein was harvested. As anticipated, moderate increases in cyclooxygenase 2 (COX2) and nitric oxide synthase 2 (NOS2) RNA expression were observed in WT. However in KO astrocytes, increases in COX2 RNA were an order of magnitude greater, and increases in NOS2 were approximately 50 fold greater than WT cells. Furthermore, significant increases in COX2 protein levels were concomitantly observed, as well as increases in prostaglandin E2. Therefore, DJ-1 KO astrocytes are highly vulnerable to LPS and respond with an exaggerated up-regulation of pro-inflammatory mediators compared to WT cells. To determine whether MAPK or the PI3K pathways contribute to alterations in COX2 and NOS2 expression, inhibitors of p38 (SB203580), MEK (U0126) JNK (JNK inhibitor), and AKT (AKT inhibitor IV) were also utilized. These data are the first to demonstrate a differential inflammatory response in mammalian cells lacking DJ-1, and provide an exciting venue of research in neurodegenerative disease.

2035 OXIDATIVE STRESS-INDUCED PHOSPHORYLATION OF PKD1 ACTIVATION LOOP IS REGULATED BY PROTEIN KINASE C-DELTA PROTEOLYTIC ACTIVATION IN CELL CULTURE MODELS OF DOPAMINERGIC DEGENERATION.

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Oxidative stress is a key mechanism by which neurotoxic chemicals induce neuronal damage; however, cell signaling mechanisms underlying oxidative neuronal damage are not well characterized. Protein kinase D1 (PKD1) belongs to a novel CaM kinase family and is emerging as an oxidative stress sensor in non-neuronal cells. We previously demonstrated that proteolytic activation of PKCδ mediates oxidative stress-induced apoptotic cell death in cell culture models of Parkinson's disease; further, phosphorylation of PKCδ Tyr311 by Src tyrosine kinase is a prerequisite for proteolytic cleavage by caspase-3. Herein, we examined whether PKCδ plays any role in activation of PKD during oxidative damage in mesencephalic dopaminergic neuronal cells (N27). Treatment of N27 cells with H_2O_2 (100-300μM) induced phosphorylation of PKD1 activation loop (Ser/Thr-744/748) in a dose- & time-dependent manner. Pretreatment with either the PKCδ inhibitor rottlerin (5μM) or p60src tyrosine-specific kinase inhibitor TSKI (5μM) almost completely abolished the H_2O_2- induced phosphorylation of PKD1. Additionally, suppression of PKCδ by siRNA significantly attenuated H_2O_2-induced phosphorylation of the PKD1 activation loop, suggesting that PKCδ indeed regulates PKD1 phosphorylation. To further determine whether the proteolytically activated PKCδ catalytic fragment mediates PKD1 phosphorylation, the pan-caspase inhibitor Z-VADfmk and N27 cells stably expressing caspase-3 cleavage-resistant PKCδ mutant (PKCδ-CRM) were used. H_2O_2-induced phosphorylation of PKD1 was significantly suppressed by Z-VADfmk (100μM), as well as in N27 cells stably expressing PKCδ-CRM mutant. Together, these results demonstrate for the first time that proteolytic activation of PKCδ regulates the phosphorylation of the PKD1 activation loop. This novel interaction between PKD1 and PKCδ may have important implications in environmental neurotoxic chemicals-induced oxidative damage in dopaminergic cells (support NIH grant ES10586 & NS45133)

2036 DEFINING "NEUROINFLAMMATION": LESSONS FROM MPTP- AND METHAMPHETAMINE-INDUCED NEUROTOXICITY.

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Neuroinflammation often is described as the underlying process responsible for toxicant-induced damage to the CNS. While glial activation and the attendant expression of proinflammatory mediators often are associated with CNS damage, it is not clear that a cause-and-effect relationship exists between the presence of a neuroinflammatory process and neural damage. We explored this issue with two models of dopaminergic neurotoxicity. We used single low-dose regimens of MPTP or METH to cause selective degeneration of striatal dopaminergic nerve terminals without affecting cell bodies in the nigra. Both compounds increased the expression of the microglia associated factors, F4/80, Il-1α, Il6, Ccl2 and Tnf-α and also elicited morphological evidence of microglial activation prior to induction of astrogliosis. Pharmacological antagonism of MPTP and METH neurotoxicity prevented these proinflammatory responses, findings suggestive of a link between neuroinflammation and the observed neurotoxic outcomes. Nevertheless, when minocycline was used to suppress the expression of all these mediators, with the exception of Tnf-α, we failed to see neuroprotection. Likewise, when we examined the effects of MPTP or METH in transgenic mice lacking Il6, Ccl2 or Tnfr1/2 genes, deficiency of either Il6 or Ccl2 did not alter neurotoxicity, whereas deficiency in Tnfr1/2 was neuroprotective. Although these observations pointed to a role of the proinflammatory cytokine, TNF-α, in the neurotoxic effects of MPTP and METH, other observations did not support this argument. For example, induction of iNOS or activation of NF-κB, effects linked to inflammatory responses and free radical formation, was not observed. Moreover, immunosuppressive regimens of glucocorticoids failed to suppress TNF-α, or block neurotoxicity. Taken together, our observations suggest that MPTP and METH neurotoxicity are associated with a "neuroinflammatory" response, yet this response lacks key features of inflammation and, with the exception of TNF-α, neurotoxicity appears to be the cause rather than the consequence of proinflammatory signals.

2037 PROLYL HYDROXYLASE INHIBITION PROTECTS AGAINST MPP+ TOXICITY *IN VITRO.*

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One prevalent feature of neurodegenerative disorders is the dysregulation of iron within different brain neurons. Compared to normal brains, iron levels are higher in affected brain areas in Parkinson's disease indicating a disruption in cellular iron regulation. Recent studies have shown that iron activates prolyl hydroxylase (PHD), which promotes the degradation of the HIF-1α, an important transcription factor that regulates pro-survival gene products. Various cellular responses were measured in dopaminergic N27 cells treated with MPP+. 2 different PHD inhibitors (DHB and DMOG) as well as an iron chelator (SIH) were able to elicit the translocation of HIF-1α into the nucleus and the up-regulation of the transferrin receptor (TfR). Additionally, sustained HIF-1α accumulation in the nucleus was observed in the presence of MPP+, suggesting that the HIF pathway is also activated in the presence of an oxidative neurotoxicant. With increasing evidence of the involvement of HIF-regulated genes encoding proteins involved in iron regulation, total iron levels were also determined. MPP+ elicited an increase in total intracellular iron of cells grown in 3% oxygen that was attenuated in the presence of DHB and DMOG. One of the factors that could contribute to the MPP+-mediated elevation in intracellular iron is the up-regulation of the TfR, responsible for the uptake of iron into cells. We observed that MPP+ resulted in a concentration-dependent increase in TfR protein levels that was significantly reduced in the presence of DHB, suggesting that the reduction in iron levels may be mediated by a down-regulation of TfR, either by the HIF or IRP pathways. To our knowledge, this study is the first to demonstrate the protective effects of PHD inhibition in a cell culture model of dopaminergic cell death associated with MPP+ toxicity. We have preliminary evidence, in both in vitro and in vivo systems, to show that the HIF pathway plays an important role in regulating proteins that are involved in the antioxidant response as well as in iron homeostasis.

2038 NUCLEAR FACTOR KAPPA B MEDIATES SELECTIVE INDUCTION OF NEURONAL NITRIC OXIDE SYNTHASE IN ASTROCYTES DURING MILD INFLAMMATORY STIMULATION WITH 1-METHYL-4-PHENYL-1, 2, 3, 6-TETRAHYDROPYRADINE.

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Recent advances in understanding the progression of Parkinson's disease (PD) implicate perturbations in astrocyte function and induction of constitutively expressed neuronal nitric oxide synthase (NOS1) in both human PD and the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyradine (MPTP) disease model. Transcriptional regulation of *Nos1* is complex, however, recent data suggest that nuclear factor kappaB (NF-KB) is an important transcription factor involved in inducible expression of the gene. The data presented here demonstrate that mild activation of astrocytes with low or 'sub-optimal' concentrations of MPTP (1 μM) and the inflammatory cytokines tumor necrosis factor alpha (10 pg/ml) and interferon gamma (1 ng/ml) results in selective induction of *Nos1* mRNA and protein, an ensuing increase in intracellular nitric oxide, and a significant elevation in global protein nitrosylation. This mild inflammatory stimulus also resulted in activation and recruitment of NF-KB to a potential NF-κB response element located in the Nos1 promoter region flanking the first exon. A role for NF-KB was confirmed through overexpression of a NF-KB "super repressor" which prevented significant induction of the NOS1. The data presented here thus demonstrate a role for NF-KB in selective induction of NOS1 during early inflammatory activation of astrocytes with low-dose MPTP and TNF-α/ IFN-γ.

2039 ALTERED DOPAMINE LEVELS AND NEURODEGENERATION IN THE SUBSTANTIA NIGRA IN RESPONSE TO INTRANIGRAL INJECTION OF ROTENONE IN MICE.

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Research suggests environmental toxins and genetic predisposition are factors in the development of Parkinson's disease, a neurodegenerative disease caused primarily by the loss of dopamine neurons in the substantia nigra. Given such evidence, we examined the potential for exposure to the toxicant rotenone on neurodegeneration of dopamine neurons in the substantia nigra of mice with a mutation in the Nurr1 gene, a genotype that makes dopamine neurons more susceptible to damage. In our current experiments, adult mice received local injections of rotenone directly into the substantia nigra and were sacrificed at various intervals of up to one week. Harvested brains were used to measure neurodegeneration via immunocytochemistry, gene eXpression profiling quantitative multiplex PCR (GeXP), and high-pressure liquid chromatography (HPLC) for catecholamine analysis. Proliferation of microglial cells, glial cells, neuron apoptosis were detected with immunocytochemistry. Multiplex GeXP was used to measure the presence of genetic responses to neural damage in the substantia nigra, with detectable levels of heme oxygenase 1, capase 3 and Bax, indicating oxidative stress and apoptosis in rotenone-treated mice. Preliminary analysis of HPLC data suggests a temporary increase in dopamine in response to rotenone-induced neurodegeneration. Initial results from these experiments suggest localized neural inflammation and neurodegeneration occurs from direct rotenone exposure in the substantia nigra.

2040 CHARACTERIZATION OF THE DIFFERENCES IN THE OXIDATIVE EVENTS IN PROTEINS IN TWO DIFFERENT PESTICIDE MODELS OF PD.

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The etiology of sporadic Parkinson's disease resides in genetic as well as environmental factors. Exposure to pesticides has been widely correlated to the progression of the disorder and chronic administration of rotenone or paraquat in animals accurately reproduces the symptoms. Even though oxidative stress is a key event underlying the action of pesticides in PD, details indicating which particular cell types are initially involved in the oxidative response are lacking so far. Remarkably, although the selective degeneration of DA neurons in the substantia nigra pars compacta is a key feature of PD, it is now clear that cell types other than neurons could participate to the pathogenesis. Here we used two new histochemical approaches to label oxidized cysteines as well as carbonyls, two of the principal modification products in proteins following oxidative insult. In both models, carbonylation appears to occur mostly outside neuronal cell bodies, at least in early stages; on the contrary, cysteines oxidation to disulfides is clearly detectable in DA neurons. These results suggest that the analyzed modifications follow similar patterns in the paraquat as well as in the rotenone model. However, as they seem to affect different cell types, they may act along different cellular pathways.

R 2041 A CASE STUDY ON THE RISKS AND BENEFITS OF DECABDE, A MAJOR BROMINATED FLAME RETARDANT.

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Decabromodiphenyl ether (DecaBDE) is a high production volume chemical used as a flame retardant in a variety of consumer products; examples of uses in high impact polystyrene and textile applications include housing of TVs, audio and video equipment, mobile phones, and upholstery textiles (e.g., sofas, office chairs). It is the only polybrominated diphenyl ether (PBDE) mixture still used commercially as the other formulations have been banned or withdrawn worldwide. However, DecaBDE is the subject of intense scientific and regulatory scrutiny given that recent studies have suggested that 1) DecaBDE is a potential developmental neurotoxicant in rodent models; 2) DecaBDE potentially degrades to lower brominated congeners which appear to be more toxic than DecaBDE itself; and 3) levels of DecaBDE appear to be increasing in humans, wildlife, and the environment. Nonetheless, some scientists suggest that despite these findings, the benefits of using DecaBDE as a flame retardant outweigh the potential health risks. This scientific roundtable will address these topics. Experts will discuss regulatory issues and status worldwide, recent toxicological findings, relative sources of exposure, biotic and abiotic degradation, and characterization of risk and associated data gaps. Finally, a cost-benefit analysis for DecaBDE will be evaluated. *(This abstract does not represent EPA policy)*

IS 2042 IMPLEMENTATION OF THE ICH S8 IMMUNOTOXICITY TESTING GUIDELINE.

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The ICH S8 Guideline, "Immunotoxicity Studies for Human Pharmaceuticals" provides recommendations for nonclinical safety testing of low molecular weight drugs for potential to induce harmful effects via suppression of the immune system. It applies to new drugs; existing drugs in which intended indication or label changes may result in unaddressed toxicological issues; and drugs in which signs of immunosuppression arise during clinical trials or following market approval. Preparation of the S8 guidance document progressed through the combined efforts of pharmaceutical industry representatives (PhRMA, JPMA, EFPIA) and members from tripartite government regulatory agencies (FDA, MHLW, CHMP) who recognized the need for globally harmonized regulatory guidance on immunotoxicological testing in drug development. Over the years leading up to the adoption of the S8 Guideline by regional regulatory agencies (2005-2006), there was significant debate and even some confusion over the compulsory versus voluntary nature of the testing recommendations and on how to apply the guidance in concert with pharmaceutical industry objectives. The purpose of this informational seminar is to provide a forum for the discussion of the practical implementation of the S8 Guideline including industry case examples and questions that remain unanswered. A panel consisting of members of the ICH S8 Expert Working Group responsible for creating the S8 Guideline and representing the U.S., Europe, and Japan will be available to answer questions from the audience. This session is intended to be an interactive forum with the expert panel, audience, and selected speakers from industry who have comments or questions on the scope and application of the S8 Guideline in current industry practice. The session will begin with a brief introduction followed by an overview of the development of the S8 Guideline and the rationale for the recommendations made. This will be followed by two individual oral presentations by industry professionals with key opinions to consider about the practical application of the Guideline, an open question and answer session, and concluding thoughts.

IS 2043 NIEHS OUTSTANDING NEW ENVIRONMENTAL SCIENTISTS (ONES) AWARDEES.

J. P. Mastin and C. Shreffler. *DERT, NIEHS, Research Triangle Park, NC.*

Concerned with assuring the quality of biomedical research, the National Institute of Environmental Health Sciences launched an initiative to support the future generation of exceptionally talented and creative new scientists. For NIEHS, this involves helping to develop a cadre of talented early-career scientists who will further the understanding of the impact of environmental exposures on human health through disciplines such as toxicology, epidemiology, and clinical research, as well as multi-disciplinary translational research. To identify outstanding scientists at the formative stages of their career and assist them in launching an innovative research program with a defined impact in the environmental health sciences, NIEHS has established the ONES program to fund R01 research grants intended for researchers who have not received their first R01 research grant. It is designed to be highly competitive, each school only being allowed to submit one application, with a limited number awarded each year. These R01s are distinguished from other R01 research grants in that they provide funding for developing necessary resources and for career development activities in addition to funding for research expenses. Research programs supported by this announcement seek to promote career advancement for new scientists who intend to make a long-term career commitment to research in the mainstream of the environmental health sciences, and bring innovative, ground-breaking research initiatives and thinking to bear on the problems of how environmental exposures affect human biology, human pathophysiology and human disease. The first round of awardees included researchers investigating the effects of air pollution on pulmonary and cardiovascular disease, the mechanisms of arsenic carcinogenesis, mechanisms of repair of environmentally induced DNA damage, and sensory neuron signaling in response to environmental exposures. This session will provide attendees the opportunity to hear the exciting, innovative research being conducted by these talented new researchers and introduce the recipients of the second round of ONES awardees.

R 2044 BIOFUEL COMBUSTION: AN EMERGING HEALTH PROBLEM?

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Recently the concept of increasing the production of liquid biofuels for energy use in the US and other countries has stirred up debate. Liquid biofuels have attracted attention as potential replacements for other liquid petroleum-based fuels primarily, but not exclusively, in on-road vehicles. Major uncertainties exist as to 1) the best biomass (e.g., corn, soy, etc.) to utilize; the net energy balance (gain or loss) in the production and transport of liquid biofuels; and 3) the market share liquid biofuels may attain. Increased biofuel production has altered land use and created ethical concerns, economic changes, and soil sustainability questions. Biofuel combustion raises additional issues as to hazard identification of the emissions produced under a wide range of conditions, such as fuel types and blends, engine types and engine operating conditions, aftertreatments, etc. To date few studies have been performed examining the health effects and biological responses with exposures to biofuel emissions. The lack of toxicological studies with biofuel emissions makes it difficult to assess the toxicity of this class of fuel relative to other petroleum-based fuels, or other potential fuels, e.g., hydrogen. This discussion session has these goals: 1) to inform the audience of the potential for different biomass types being incorporated into the global energy structure; 2) describe emission products of biofuel combustion, with an emphasis on biodiesel exhaust as one of the better studied biofuels. Comparison to emissions from petroleum diesel engines will allow assessment as to how similar the emissions may be in terms of toxic components, assisting in risk assessment for biodiesel exhaust; 3. to present an overview of the known toxicology of biofuel emissions, with reference to petroleum diesel exhaust literature, to identify areas where assessment of the effects of biofuel emissions exposure should be pursued. [This abstract may not reflect EPA policy.]

2045 MODULATION OF CELLULAR ANTIOXIDANT ENZYMES ACTIVITIES AND GLUTATHIONE LEVEL BY THE DRINKING WATER CHLORINATION BY-PRODUCT, DICHLOROACETATE.

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Dichloroacetate (DCA) is one of the most toxic haloacetates by-products produced during the process of water chlorination. DCA was found to cause concentration- and time-dependent production of superoxide anion (SA), associated with cellular

death when tested in J774A.1 cell culture. In this study, the DCA effects on the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-PX), as well as glutathione (GSH) level were tested in J774.A1 macrophage cells. Groups of cell cultures were incubated with 24 mM of DCA for 24 and 48 h, and cells were then collected and assayed for SOD, CAT and GSH-PX activites and GSH level. While addition of DCA to the cells resulted in approximately 50% and 95% cellular death after 24 and 48 h incubation, respectively, the activities of SOD, CAT and GSH-PX were significantly increased among the viable cells at both time points. Also, significantly more increases in the activities observed after incubation for 48 h, as compared with the 24 h incubation time. GSH levels on the other had were significantly suppressed among the viable cells, with significantly more suppression observed at the 48 h time point, as compared with the 24 h time point. Addition of glutathione at concentrations ranging between 15-60 nmole/ml to the DCA-treated cells resulted in concentration-dependent increases in the number of viable cells, with maximal effects achieved with GSH concentration of 45 nmole/ml. It is concluded that viable cells are partially protected against DCA-induced cellular death because of increased antioxidants enzymes activities in response to the compound, but later cellular death occurs because of GSH depletion.

2046 INCREASED MITOCHONDRIAL THIOREDOXIN POTENTIATED N-ETHYLMALEIMIDE-INDUCED CYTOTOXICITY.

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The antioxidant and antiapoptotic functions of mitochondrial thioredoxin (Trx2) are dependent on the conserved cysteines (Cys) at its redox active center. In the current study, we showed by mass spectrometry that a thiol alkylating compound, NEM, could directly modify Cys 93 of Trx2. The interaction between NEM and Trx2 in intact cells was confirmed by redox Western analysis. Overexpression of Trx2 in cultured 143B osteosarcoma cells caused increased sensitivity to NEM. Such sensitization was not observed in cells overexpressing the C93S mutant of Trx2. Thus, covalent modification of the Cys93 will inhibit the dissociation between Trx2 and its substrate proteins and will likely result in a dominant-negative effect in response to environmental toxicities. (Supported by NIH grants ES09047 and ES014668)

2047 NRF2-DEPENDENT ANTIOXIDANT SYSTEM AND PI3 KINASE/AKT PATHWAY IN ASTROCYTES EXPOSED TO METHYLMERCURY (MEHG).

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Mercury (Hg) compounds have high affinity with the sulfhydryl group. Consequently, cellular glutathione (GSH) forms a primary line of defense against MeHg toxicity, binding intracellular Hg and preventing its association with other sensitive targets. Astrocytes are a major site of lesion in MeHg-induced neurotoxicity and the GSH synthesis in these cells is largely controlled by nuclear factor erythroid 2-related factor 2 (Nrf2). In the present study, we showed that MeHg exposure activated Nrf2 and upregulated its downstream antioxidant genes in primary rat neonatal astrocytes. Cells treated with wortmannin and LY294002 showed decreased Nrf2 activity and decreased levels of GSH. MeHg did not affect the activation of the phosphatidylinositol 3 (PI3) kinase; however, PI3 kinase inhibitors potentiated high dose mercury-induced cell death. The functional interaction between the two signaling pathways underlies an important mechanism of protecting astrocytes against MeHg toxicity. Modulation of Nrf2 by pharmacological modalities should afford a treatment to attenuate MeHg-induced neurotoxicity. (Supported by NIH grants ES07331 and ES014668)

2048 MILD ENDOPLASMIC RETICULUM (ER) STRESS PRECONDITIONING ATTENUATES METHYLMERCURY (MEHG)-INTOXICATION IN MYOGENIC CELL LINE.

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Cellular stress responses following MeHg exposure were investigated using myogenic cell lines that showed different susceptibilities to MeHg in order to find protective factors against MeHg-intoxication. The susceptible cell line showed apoptosis within 24 h after exposure to low levels of MeHg. The activation of caspase 12, 9, and 3 suggests that MeHg causes apoptosis via both mitochondria- and ER-generated processes. An early increase in the level of intracellular reactive oxygen species (ROS) was quantitatively recognized in both MeHg-susceptible and non-susceptible cell lines; however, the increase was lower in the latter cell line. The phosphorylation of apoptosis signal-regulating kinase 1 (ASK1) was also recognized in both cell lines, with the increase in intracellular ROS. However, the activation of stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) pathways was observed only in the MeHg-susceptible cell line. In contrast, the non-susceptible cell line exhibited activation of the cell survival ERK pathway. Up-regulation of metallothioneine I (MTI) and Hic-5 mRNAs encoding proteins induced by oxidative stress was recognized during the early stage of MeHg cytotoxicity. Real-time PCR and western blot analyses confirmed that ER stress is a late event during MeHg cytotoxicity. Coaddition of the antioxidant Trolox dramatically suppressed the increase in the level of ROS, activation of caspases and, finally, apoptosis. However, later treatment with Trolox attenuated its protective effect against MeHg cytotoxicity. Pre-treatment with low concentration of thapsigargin (a specific inhibitor of ER Ca^{2+}-ATPase) decreased subsequent MeHg-cytotoxicity. The prior mild ER stress induced high expression of ER chaperone GRP 78 and stress protein MTI mRNAs, and rendered cells resistant to cell death. The results indicate that failure to protect cells against the early oxidative stress triggers ER stress and apoptosis processes. Mild ER stress preconditioning attenuates MeHg-intoxication through upregulation of GRP78 and MTI.

2049 INCREASED OXYGEN UTILIZATION AND OXIDATIVE STRESS IN CHO CELLS DURING DIQUAT REDOX CYCLING.

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Acute exposure to diquat, a non-specific bipyridyl-class defoliant, causes injury to many tissues including the lung, liver, kidney and brain. Tissue injury is mediated by enzyme catalyzed redox cycling whereby diquat is reduced by NADPH-dependent oxidoreductases. The rapid reaction of reduced diquat with oxygen regenerates diquat and produces reactive oxygen intermediates (ROI). Cytochrome P450 reductase is a key enzyme mediating this process. In the present studies, we compared diquat redox cycling in a Chinese hamster ovary cell line expressing 30-fold more cytochrome P450 reductase (CHO-OR) than wild type control cells (CHO-WT). Diquat (100 μM) readily redox cycled in lysates from both cell types producing superoxide and hydrogen peroxide. Lysates from CHO-OR cells produced 10-30 fold more ROI than CHO-WT cells. Using self-referencing oxygen and hydrogen ion microsensors, the effects of diquat on metabolic activity of intact CHO cells in monolayer culture were investigated. Diquat (10-100 μM) was found to markedly increase oxygen (2-3 fold), but not hydrogen ion flux, in both CHO-WT and CHO-OR cells. In the presence of 10 μM diquat, oxygen utilization was 50% greater in CHO-OR cells. The mitochondrial poisons cyanide (1 mM), rotenone (1 μM), and antimycin A (100 μM) prevented mitochondrial, but not diquat, mediated oxygen flux into cells. In contrast, diphenyleneiodonium (10 mM), an NADPH oxidase inhibitor, blocked the effects of diquat without altering mitochondrial respiration. These data indicate that redox cycling and increased oxygen utilization by diquat are important in inducing oxidative stress. Increased oxidative stress in CHO-OR cells suggests that these processes are mediated by cytochrome P450 reductase. Supported in part by NIH grants ES05022, AR055073, CA100994 and CA093798.

2050 EFFECT OF TWO NITROSOUREA DERIVATIVES ON THE ANTIOXIDATIVE DEFENSES OF NEOPLASTIC CELLS.

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Carmustine – nitrosourea derivative, is frequently used as anticancer drug. High toxicity and low selectivity of carmustine are the major reasons for a limited use of this antineoplastic therapy. Therefore, it is important to find new compounds that have improved therapeutic index. -N-[N`-(2-bromophenolo)-N`-nitrosocarbamoylo]-proline analog
(AC) was synthesized. The objective of this research was to evaluate the influence of carmustine and proline analogue of nitrosourea (100 micro molar) on the antioxidant abilities of fibroblasts and leukemia cells. Activity of antioxidant enzymes and the concentrations of glutathione and lipids peroxidation product malondialdehyde (MDA) were determined.
Overall, the activity of antioxidant enzymes was decreased

after carmustine and AC addition in normal and neoplastic cells. AC caused, the most remarkably, by about 85%, diminution in Cu, Zn- superoxide dismutase (SOD) activity and completely inhibited Mn-SOD activity in MOLT4 cells. However, carmustine more significantly decreased activity of Cu, Zn-SOD and Mn-SOD in fibroblasts (by about 60% and
70%, respectively). AC caused significant decrease in the activity of glutathione reductase and glutathione peroxidase in MOLT4 cells (by about 100% and 67%, respectively), while carmustine reduced the activity of above enzymes by about 37% and 55% respectively. The level of glutathione was
reduced in MOLT4 cells by about 33% after AC addition, while carmustine caused an increase in the level of glutathione in fibroblasts by about 35%. Inhibition in the antioxidative parameters were accompanied by increase in lipids peroxidation products. Increase in MDA concentration was observed after AC administration both in fibroblasts (by about 12%) and in MOLT4 cells (by about 20%). Carmustine caused an increase in MDA concentration by about 26% in fibroblasts and by about 10% in MOLT4 cells. In conclusion, these results suggest that differences in inhibition of antioxidant defense system in neoplastic cells can be further used to improve the antineoplastic activity of these two agents.

2051 THE ROLE OF GLUTATHIONE IN 3*H*-1, 2-DITHIOLE-3-THIONE-MEDIATED CYTOPROTECTION AGAINST ACROLEIN-INDUCED NEUROCYTOTOXICITY.

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Acrolein, an unsaturated aldehydic product of lipid peroxidation has been implicated in the pathogenesis of several neurodegenerative disorders including Parkinson's and Alzheimer's diseases. While a number of cellular factors including glutathione (GSH), GSH S-transferase (GST) and aldose reductase (AR) have been proposed to be involved in the detoxification of acrolein in target cells, whether chemical upregulation of these cellular defenses affords protection against acrolein toxicity in neuronal cells has not been investigated. In this study, we have characterized the inducibility of GSH, GST and AR by 3*H*-1,2-dithiole-3-thione (D3T) and the protective effects of the D3T-elevated cellular defenses on acrolein-mediated toxicity in human neuroblastoma SH-SY5Y cells. Incubation of SH-SY5Y cells with D3T resulted in a marked concentration- and time- dependent induction of GSH, but not GST or AR. Pretreatment of SH-SY5Y cells with D3T led to a dramatic decrease of acrolein-induced cytotoxicity, as assessed by MTT reduction assay and phase-contrast microscopy. Incubation of SH-SY5Y cells with acrolein for 0.5 h and 1 h also resulted in a significant depletion of cellular GSH, which preceded the decrease of cell viability. To further demonstrate the involvement of GSH in protecting against acrolein-induced cytotoxicity, buthionine sulfoximine (BSO) was used to inhibit cellular GSH biosynthesis. Co-treatment of SH-SY5Y cells with BSO and D3T was found to completely block the D3T-mediated GSH induction and to largely reverse the cytoprotective effects of D3T on acrolein-induced toxicity. Taken together, this study demonstrates for the first time that the D3T-augmented cellular GSH defense affords a marked protection against acrolein-induced neuronal cell injury.

2052 OFLOXACIN-INDUCED INJURIES OF JUVENILE RABBIT CHONDROCYTES IN CULTURE THROUGH OXIDATIVE STRESS.

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Quinolones (QNs) belong to chemosynthesis antibacterials. Due to its broad antibacterial spectrum and good antibacterial activity, QNs has been applied for the infection therapy widely. However, QNs-induced arthropathy of immature animals has led to restrictions on the therapeutic use of these antimicrobial agents. The exact mechanism(s) of QNs-induced chondrotoxicity remain unknown. The present study was undertaken to investigate the potential oxidative damage of ofloxacin (one typical QNs) to Juvenile rabbit joint chondrocytes in culture. Chondrocytes were incubated with ofloxacin at concentrations of 0, 5, 10, 20, 40 and 80 μg/ml respectively, the extent of oxidative damage was assessed by measuring reactive oxygen species (ROS), lipid peroxidation and antioxidant defense system. It was observed that ofloxacin resulted in a significant lipid peroxidation in chondrocytes, revealed by a concentration-dependent increase in the level of thiobarbituric acid reactive substances (TBARS). Similarly, this drug induced a concentration-dependent increase in intracellular ROS production measured by spectrofluorimetric method using 2,7-dichlorodihydrofluoresceindiacetate (DCHF-DA) fluorescent probe. Furthermore, the activity of antioxidant enzymes in chondrocytes, such as glutathione peroxidase (GSH-px), catalase (CAT), superoxide dismutase (SOD), were rapidly decreased after treatment with ofloxacin. Especially, it was found that the activity of GSH-px was decrease by 17.47%, 19.16%, 51.10%, 67.70%, 69.14% with ofloxacin at concentrations of 0, 5, 10, 20, 40 and 80 μg/ml, respectively. In addition, SOD inhibition and ROS production was strongly inhibited, and the loss in cell viability was partly abated by thiol-containing compounds GSH, N-acetylcysteine (NAC) and dithiothreitol (DTT). In conclusion, these results clearly demonstrate that oxidative stress plays an important role in ofloxacin - induced injuries of Juvenile joint chondrocytes. Supported by China Natural Science Foundation grant 30500641.

2053 THE EFFECT OF THE LIPID PEROXIDATION PRODUCT 4-HYDROXY-2-NONENAL ON ESTERASE AND LIPASE ACTIVITIES IN HUMAN THP-1 MONOCYTES/MACROPHAGES.

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Lipid peroxidation frequently occurs as a result of oxidative stress. Several electrophilic aldehydes are formed when lipid peroxides break down, one of the most prominent being 4-hydroxy-2-nonenal (4-HNE). 4-HNE has a long enough half life to allow diffusion throughout the cell before covalently interacting with proteins. Cellular proteins covalently modified by 4-HNE may be inactivated, thereby contributing to diseases such as atherosclerosis. Here, we examined the effect of 4-HNE on esterase and lipase activities in cultured human THP-1 monocytes/macrophages. These enzymes are important regulators of cholesterol mobilization. Dysregulated cholesterol mobilization from monocytes/macrophages contributes to phenotypic differentiation into 'foam cells' and subsequent atherosclerosis. The following results were obtained. First, when pure recombinant human carboxylesterase 1 was treated with increasing amounts of 4-HNE, significant inhibition of hydrolytic activity was observed. The molecular mechanism for this inactivation is currently unknown. Second, when THP-1 cell lysates were treated with 4-HNE, marked decreases in esterase and lipase activities were also noted. Third, when intact THP-1 cells in culture were treated with 4-HNE (25-200 micromolar) for up to 24 hours, decreased esterase and lipase activities were observed after normalization with total protein though some cytotoxicity was noted. Moreover, western blotting indicated induction of carboxylesterase 1 protein in cells treated with 200 micromolar 4-HNE. This may indicate a compensatory response. Collectively, these results suggest that pathophysiological concentrations of 4-HNE may lead to inhibition of enzymes involved in processing lipid and water-soluble esters. [Supported by NIH 5P20RR17661, trainee stipend supported by NIH-NCRRRR070710]

2054 CYANIDE NEUROTOXICITY: INVOLVEMENT OF UCP-2 UPREGULATION, MITOCHONDRIAL GLUTATHIONE DEPLETION AND BNIP3.

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Uncoupling protein 2 (UCP-2), a member of the mitochondrial proton transport family, is located in inner mitochondrial (MT) membrane. UCP-2 overexpression enhances cyanide-induced neurotoxicity by further reducing MT membrane potential ($\Delta\Psi m$) and ATP synthesis. However the molecular mechanism by which cyanide initiates neuronal cell death is not clear. The purpose of this study was to determine whether BNIP3, a pro-death Bcl-2 protein, is involved in the UCP-2 enhancement of cyanide neurotoxicity. To overexpress UCP-2, N27 immortalized mesencephalic cells were transiently transfected with UCP-2. In UCP-2 overexpressed cells (UCP-2+), cyanide markedly decreased MT glutathione (mtGSH) and upregulated BNIP3 expression. Restoration of GSH with glutathione ethyl ester (GSH-EE) or N-acetyl-L-cysteine (NAC) attenuated cyanide mediated mtGSH depletion and the BNIP3 upregulation. To determine whether the induction of BNIP3 expression by cyanide is mediated by mtGSH depletion, UCP-2+ cells were treated with ethacrynic acid (EA), a glutathione S-transferase inhibitor. EA induced a rapid depletion of mtGSH, followed by BNIP3 up-regulation. Like cyanide, EA also produced cell death in UCP-2+ cells. Knockdown of BNIP3 by RNAi in UCP-2+ cells attenuated the decrease of $\Delta\Psi m$ and cell death produced by cyanide. Since it was apparent that BNIP3 was a mediator of cyanide neurotoxicity, the mechanism of its induction was then determined. After cyanide or EA treatment, mitochondrial generation of superoxide was markedly increased, followed by accumulation of hypoxia-inducible factor-1α (HIF-1α), a transcription factor which regulates BNIP3 expression. It is concluded that cyanide-induced mtGSH depletion leads to an increased MT oxidative stress, which stimulates HIF-1α accumulation and then BNIP3 induction. The increased expression of BNIP3 then mediates cyanide-induced neurotoxicity. (This work was supported by NIH grant ES 04140)

2055 CATALASE AMELIORATES POLYCHLORINATED BIPHENYL-INDUCED CYTOTOXICITY IN HUMAN BREAST EPITHELIAL CELLS.

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Polychlorinated biphenyls (PCBs) are environmental chemical contaminants believed to adversely affect cellular processes. We investigate the hypothesis that PCB-induced changes in cellular reactive oxygen species (ROS) levels induce DNA damage resulting in cytotoxicity. Asynchronously growing exponential cultures of human non-malignant breast epithelial cells (MCF10A) were incubated with PCBs for 3d and assayed for cell growth, DNA damage, cellular ROS levels, and cytotoxicity. 2-(4-chlorophenyl)-1,4-benzoquinone (BQ)
and 2, 2', 4, 4', 5, 5'-hexachlorobiphenyl (PCB153) exposures significantly decreased cell number, MTT reduction, and increased the percentage of cells with sub G1 DNA content. Results from electron paramagnetic resonance spectroscopy showed approximately 4-fold increase in ROS levels, which was suppressed in cells treated with catalase. Increased ROS levels were associated with decreased cell survival, which was partially rescued in cells pretreated with PEG-catalase. A dose dependent increase in micronuclei frequency was observed in 3, 3', 4, 4'-tetrachlorobiphenyl (PCB77), PCB 153 and BQ treated cells. These results were consistent with an increase in histone 2AX-phosphorylation. Interestingly, treatment of cells with PEG-catalase blunted PCB-induced increase in micronuclei frequency and H2AX phosphorylation that was consistent with an increase in clonogenic cell survival. Our results showed PCB-induced increase in cellular ROS levels caused DNA damage resulting in cell killing. Treatment with catalase decreased PCB-induced cytotoxicity. These results suggest the feasibility of a redox-based therapy protocol for PCB-exposed individuals.

2056 PARADOXES OF MNSOD OXIDATIVE MODIFICATION: A NEW PROTECTIVE FUNCTION AGAINST OXIDATIVE STRESS.

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Exposure of cells to toxic chemicals and pro-oxidants generates a range of harmful reactive oxygen (ROS) and nitrogen (RNS) species. When ROS and RNS overwhelm the cellular defenses (antioxidant enzymes, scavengers), they cause oxidative/nitrosative stress, damage and cell death. Manganese superoxide dismutase (MnSOD) is a key mitochondrial enzyme that controls and prevents toxic action of ROS and RNS. Excessive production of ROS and RNS has been associated with a loss of catalytic activity of MnSOD - supposedly due to modification of its tyrosine residues - leading to perpetual oxidative stress. Consequently, overexpression of MnSOD (by delivery of plasmid/liposome complexes) delays the onset and reduces the extent of oxidant-induced cell injury. We found that oxidants induced cross-linking of MnSOD subunits via mechanism of tyrosine dimerization rendering its 3D structure substantially more rigid. Oligomeric MnSOD - with cross-linked dityrosines - preserved catalytic activity and was resistant to unfolding, degradation and peroxynitrite mediated inactivation. Paradoxically, oxidation of MnSOD stabilized the enzyme and favorably affected its potency in mitochondrial antioxidant protection. This suggests that oxidative stress can be used as a promising regimen for pre-conditioning-based protection of cells and tissues against damage caused by MnSOD-deficiency. We report that short pretreatment (30 min) of mouse embryonic cells with oxidative stress inducing agents (antimycin A, rotenone, hydrogen peroxide) increased their resistance to subsequent oxidative/nitrosative stress. This was accompanied by accumulation of MnSOD oligomers. Based on comparative experiments with wild type cells and MnSOD-/- cells, we discuss the role of MnSOD oligomerization as a mechanism of instant adaptation contributing to increased resistance of cells to oxidative stress. Supported by NIH U19A1068021.

2057 IDENTIFICATION OF CELLULAR PHENOTYPES, MECHANISMS AND PATHWAYS OF RADIOSENSITIVITY IN HUMAN CELL LINES.

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The importance of an appropriate response to DNA damage by ionizing radiation (IR) is reflected in the radiosensitivity, cancer susceptibility, and other devastating consequences of the inherited chromosomal instability syndromes, such as Ataxia-Telangiectasia (A-T), Nijmegen Breakage Syndrome, Mre11 deficiency, Fanconi Anemia (FA) and DNA Ligase IV deficiency. These disorders serve as the prototypes for unraveling the genetics of sensitivity to ionizing radiation. We are dissecting the molecular mechanisms underlying radiosensitivity using a unique panel of immortalized human lymphoblastoid cell lines (LCLs) that are radiosensitive (RS-LCLs) for unknown reasons. We performed functional assays: 1) Colony Survival Assay (CSA), 2) Radioresistant DNA synthesis, (RDS), 3) In vitro plasmid-based assay to assess DNA end-joining, and 5) cytogenetics, to subset a panel of 20 RS LCLs according to cellular and molecular pathways. All 20 cell lines were RS by CSA. Of these, 13/20 had an RDS phenotype indicative of a defective intra-S phase checkpoint; while 3/20 were defective for in vitro DNA end joining. We found that 17/20 had some form of chromosomal aberrations. These findings suggest that radiosensitivity can result from genetic defects in cell cycle checkpoints or DNA end joining of double strand breaks and allow us to focus future studies on these mechanisms.

2058 MITOCHONDRIAL MECHANISMS OF ROS PRODUCTION AND OXIDATIVE STRESS BY REDOX CYCLING HERBICIDES IMPLICATED IN PARKINSON'S DISEASE.

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Exposure to environmental pesticides causes significant brain damage and has been linked with the etiology of Parkinson's disease (PD). Bipyridyl herbicides, such as paraquat (PQ) and diquat (DQ) and benzyl viologen (BV), are redox cycling agents (RCAs) known to exert cellular damage through the production of reactive oxygen species (ROS) leading to oxidative stress. However, the site and mechanism of action within the brain of these compounds has not been resolved. The goal of this study was to determine the mechanisms by which redox cycling compounds exert neurotoxicity. Previous work in our laboratory has shown that mitochondria are a major cellular site for PQ-induced H2O2 production. Here, we further characterized the role of mitochondria in redox cycling-induced ROS production and determined if in vivo treatment with PQ results in mitochondrial oxidative stress. Following treatment with redox cycling agents, H2O2 production was attenuated in respiration-deficient SH-SY5Ydopaminergic cells compared to controls. In isolated rat brain mitochondria, DQ and BV stimulated H2O2 production at rates significantly greater than PQ at similar concentrations. RCA-induced ROS production was inhibited by antimycin A suggesting a role for Complex III of the mitochondrial respiratory chain. Additional mechanistic studies further identified the Qi site of cytochrome b within Complex III as the potential site of ROS production by these RCAs. Administration of 10 mg/kg PQ biweekly for three weeks significantly increased mitochondria DNA damage assessed by qPCR in PQ-treated mice. Additionally, reduced coenzyme A, an index of the mitochondrial redox status was significantly decreased in the ventral midbrain of PQ-treated mice in comparison with saline-treated mice. These data provide evidence that bipyridyl herbicides are capable of using mitochondria as a site for redox cycling to generate ROS and emphasize the importance of further identifying mitochondrial mechanisms by which environmental agents produce oxidative stress and parkinsonism.

2059 ENVIRONMENTAL AND LIPID PEROXIDATION DERIVED ALDEHYDES INDUCE ENDOPLASMIC RETICULUM STRESS AND UNFOLDED PROTEIN RESPONSE IN VASCULAR SMOOTH MUSCLE AND ENDOTHELIAL CELLS.

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Environmental aldehydes (e.g. acrolein) and aldehydes derived from oxidation of phospholipids [e.g., 1-palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphocholine – POVPC and 4-hydroxy, trans-2-nonenal – HNE] are highly atherogenic. Although the mechanisms of their actions are unclear, such aldehydes increase cell adhesion, stimulate cytokine production and up regulate scavenger receptors involved in lipid uptake. We found that incubation of vascular smooth muscle cells (SMC) with HNE (50 μM) resulted in the modification of several endoplasmic reticulum (ER) - proteins including Grp 78, Grp 94 and PDI. Incubation of SMC and endothelial cells (EC) with acrolein, HNE and POVPC (10-50 μM) increased the phosphorylation of eIF2α (eukaryotic initiation factor-2α) by 1.5-3.0 fold and induced the transcription factors ATF3 and ATF4 (proteins) by 3-10 fold. POVPC also activated JNK by 3-6 fold in EC. Pharmacological inhibition of JNK inhibited the aldehyde-induced induction of ATF3 and ATF4 proteins by 70-90 % but not the phosphorylation of eIF2α. Treatment with the chemical chaperone, phenyl butyric acid (PBA), diminished aldehyde-induced expression of ATF3 and ATF4 proteins and phosphorylation of JNK by 80-90 %. Incubation of EC with these aldehydes

also increased the adhesion of THP-1 cells (monocytes) to EC by 1.4-1.6 fold. Moreover, incubation of EC with POVPC increased the mRNA level of the pro-inflammatory cytokine IL-8 by > 25 fold, and POVPC and HNE caused the induction of the pro-apoptotic protein, CHOP. PBA diminished aldehyde-induced endothelial cell-monocyte adhesion, IL-8 formation and CHOP induction by 60-95%. These data suggest that aldehydes activate vascular cells in part by inducing ER-stress and the inflammatory signaling could be attenuated by chemical chaperones of protein folding. This work supported by PO1 ES11860 and HL59378.

2060 PROCAINAMIDE, BUT NOT N-ACETYLPROCAINAMIDE, INDUCES PROTEIN RADICAL FORMATION ON MYELOPEROXIDASE: A POTENTIAL MECHANISM OF AGRANULOCYTOSIS.

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Procainamide (PA) is an antiarrythmic drug which normalizes heart rhythms. However, a serious side-effect associated with this drug is the 1-4% risk of agranulocytosis (severe neutrophil depletion). This condition renders the patient susceptible to infection with a 10% mortality risk. The mechanism of agranulocytosis is unknown, but reactive drug metabolites have been proposed as possible mediators. Our research focuses on free radical drug metabolites as potential mediators of agranulocytosis. Myeloperoxidase (MPO) is an enzyme capable of drug metabolism that is present in neutrophils. Therefore, we have investigated the formation of free radicals on this enzyme as a mechanistic basis of PA-induced agranulocytosis. Reactions containing PA, MNP, MPO, and H_2O_2 showed that a phenyl radical metabolite was formed. We replaced MNP with DMPO and identified the same metabolite as well as an *N*-centered radical. *N*-Acetyl-PA (NAPA) formed less radical metabolites. Protein radicals were detected by ELISA using an antibody that recognized DMPO-protein adducts. Reactions containing PA, H_2O_2, DMPO, MPO resulted in an MPO-DMPO adduct, which was inhibited by MPO inhibitors, ascorbate, or replacement of PA with NAPA. We tested PA in MPO-rich HL60 cells to form protein radicals. After incubation of the cells with DMPO, PA, & glucose oxidase (generates H_2O_2), protein-DMPO adducts were formed. We found that MPO-DMPO adducts were formed by isolating MPO via affinity purification. We confirmed these findings by ESR spectrometry using HL60 cell lysate with PA, MNP, and H_2O_2, which showed a spectrum with broad peaks indicative of protein radical formation; the spectrum was decreased by replacing PA with NAPA. MOPAC calculations showed that PA has a lower ionization potential than NAPA, which may account for the latter forming fewer metabolites and less protein radicals. We plan to investigate the toxicological role of an MPO free radical formed in neutrophils in order to determine its role in drug-induced agranulocytosis.

2061 OXIDANT-INDUCED ARACHIDONIC ACID OXIDATION AND RELEASE FROM ISOLATED RABBIT RENAL MITOCHONDRIA: ROLE OF $iPLA_2\gamma$

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Ferrous iron (Fe^{+2}) causes oxidation of membrane lipids, including those in mitochondria (MITO). We demonstrated that MITO calcium-independent phospholipase A_2 ($iPLA_2\gamma$) is protective during oxidant stress. The goal of this study was to identify the role of $iPLA_2\gamma$ in the turnover of MITO phospholipids (PL) and fatty acids during oxidative stress. Isolated rabbit renal MITO were incubated in the presence or absence of Fe^{+2} sulfate (50μM). Once Fe^{+2}-induced MITO swelling began, a marker of increased inner membrane permeability, MITO and media were collected, lipids extracted and analyzed by electrospray ionization-mass spectrometry to identify alterations in PL and fatty acids. Treatment of MITO with Fe^{+2} decreased PL containing arachidonic acid (AA) (16:0-20:4, 18:0-20:4, 18:1-20:4) and increased 16:0-lysophosphatidylcholine (LPC). AA release was greater in Fe^{+2}-treated MITO than controls, while levels of myristic, palmitic, and oleic acids did not change. The oxidized AA metabolites mono- and tris-hydroxy ($(OH)_3$)-AA also were released into the medium of Fe^{+2}-treated MITO. To determine if iPLA2γ mediates release of oxidized or unoxidized AA, the $iPLA_2$ inhibitor bromoenolactone (BEL) or diluent was added to MITO. BEL alone or in the presence of Fe^{+2} did not affect 16:0-20:4, 18:0-20:4, 18:1-20:4 PL or 16:0-LPC. However, BEL decreased Fe^{+2}-mediated AA release into the medium and potentiated Fe^{+2}-mediated $(OH)_3$-AA release into the medium. In conclusion, MITO swelling induced by Fe^{+2} is associated with release of AA and oxidized AA and decreases in PL containing AA. The observation that inhibition of $iPLA_2\gamma$ increases Fe^{+2}-mediated AA oxidation supports the hypothesis that $iPLA_2\gamma$ functions as part of a MITO phospholipid repair mechanism. In contrast, the observation that $iPLA_2\gamma$ inhibition blocked AA release supports the hypothesis that AA release does not contribute to MITO swelling.

2062 $iPLA_2\gamma$ PREVENTS OXIDANT-INDUCED LIPID PEROXIDATION AND Ca^{2+} RELEASE IN ISOLATED RABBIT KIDNEY CORTEX MICROSOMES.

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We have previously shown that calcium-independent phospholipase $A_2\gamma$ ($iPLA_2\gamma$), present in both endoplasmic reticulum (ER) and mitochondria, protects renal proximal tubule cells (RPTC) from oxidant-induced lipid peroxidation and necrotic cell death. We hypothesized that ER-$iPLA_2\gamma$ prevents and/or mediates repair of oxidant-induced ER lipid damage, and, thereby, prevents the loss of ER Ca^{2+} homeostasis, an important trigger of necrotic cell death. We investigated the role of $iPLA_2\gamma$ in oxidant-induced lipid peroxidation and Ca^{2+} release in isolated rabbit kidney cortex microsomes. Cis-parinaric acid, a naturally fluorescent lipid that loses fluorescence when oxidized, was incorporated into microsomes. Microsomes were treated with model oxidant *tert*-butylhydroperoxide (TBHP) in the presence and absence of $iPLA_2\gamma$ inhibition with bromoenol lactone (BEL) or antioxidant, butylated hydroxyanisole (BHA). TBHP induced lipid peroxidation, BHA prevented the lipid peroxidation, and $iPLA_2\gamma$ inhibition potentiated the lipid peroxidation. We developed an assay to monitor ER Ca^{2+} release using the membrane impermeant Ca^{2+} indicator, fluo-4. After ATP-dependent Ca^{2+} loading, microsomes were treated with thapsigargin, an ER Ca^{2+} pump inhibitor, to prevent further Ca^{2+} loading and then treated with TBHP in the presence and absence of BEL or BHA. TBHP caused ER Ca^{2+} release after the initiation of lipid peroxidation, $iPLA_2\gamma$ inhibition potentiated the ER Ca^{2+} release and BHA attenuated the TBHP-induced Ca^{2+} release. We suggest that ER-$iPLA_2\gamma$ plays a protective role in oxidant-induced necrotic cell death by decreasing oxidant-induced ER lipid peroxidation, and, thereby, preventing loss of ER membrane integrity and Ca^{2+} release.

2063 DEVELOPMENT OF A DOMINANT-INHIBITORY MUTANT OF THE CATALYTIC SUBUNIT OF GLUTAMATE CYSTEINE LIGASE.

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Mammalian cells possess a wide range of antioxidant defense mechanisms to counteract the deleterious effects of oxidative stress. The tripeptide antioxidant glutathione (GSH) plays a central role in protecting against oxidative injury. The rate-limiting enzyme in GSH biosynthesis is glutamate cysteine ligase (GCL), which is a heterodimeric holoenzyme composed of a catalytic (GCLC) and a modifier (GCLM) subunit. While pharmacological inhibitors of GCLC, such as buthionine sulfoximine (BSO), are routinely employed to assess the importance of GSH biosynthesis in dictating cellular responses to oxidative stress, we and others have identified several non-specific side-effects associated with the use of BSO. In an attempt to develop a more specific means of inhibiting GSH biosynthesis, we created two dimerization-competent, enzymatically-deficient GCLC mutants and examined their ability to function in a dominant-inhibitory manner by competing with wild-type GCLC for heterodimerization with GCLM, preventing formation of functionally active GCL holoenzyme. Two previously identified inactivating mutations of GCLC were employed for these studies. A naturally occurring human mutation GCLC(P158L), which possesses 2% of wild-type activity, and an artificial trypanosome mutation GCLC(E103A), which is completely inactive. We introduced these two mutations into murine GCLC and assessed their effect on enzymatic activity, ability of heterodimerization with GCLM, and ability to function as a dominant-inhibitory GCLC mutant in vitro. Interestingly, while neither mutation affected the ability of the GCLC mutant to heterodimerize with GCLM, only the E103A mutation rendered murine GCLC enzymatically inactive. Importantly, the GCLC(E103A) mutant functioned to inhibit wild-type GCL holoenzyme activity in a dose-dependent manner. The ability of GCLC(E103A) to function in a dominant-inhibitory manner in vitro supports this approach as a viable means to selectively suppress cellular GSH biosynthetic capacity.

2064 CATALASE ABROGATES POLYCHLORINATED BIPHENYLS-INDUCED DELAYS DURING QUIESCENT HUMAN BREAST EPITHELIAL CELLS' ENTRY INTO THE PROLIFERATION CYCLE.

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Polychlorinated biphenyls (PCBs) are environmental chemical contaminants believed to adversely affect cellular processes. PCBs and their metabolites are known to produce reactive oxygen species (ROS) by autoxidation of dihydroxy PCBs or by

reduction of quinones and redox cycling. We investigate the hypothesis that PCB induced ROS-signaling regulates G0/G1 (quiescent or confluent) cells' entry into S-phase (proliferation cycle). Monolayer cultures of quiescent human non-malignant breast epithelial cells (MCF10A) were incubated with 0-3 micromolar of PCBs for 4d, and re-plated at a lower cell density. Cells were harvested at the time of re-plating and various times thereafter for flow cytometry assays of cell cycle positions, ROS levels, and antioxidant enzyme activities. PCB (4-chloro-1, 4-benzoquinone (BQ), 2, 2', 4, 4', 5, 5'-hexachlorobiphenyl (PCB153), and aroclor 1254) treated quiescent cells exhibited a dose-dependent delay entering S-phase following re-plating, and the delay was maximal in 3 micromolar BQ-treated cells. In contrast to BQ treated exponential cultures, BQ treated quiescent cells showed 2-fold decrease in MnSOD and catalase activities, which correlated with increase ROS levels. The decrease in MnSOD activity was associated with decrease in MnSOD protein levels. Quiescent cells treated simultaneously with BQ and PEG-catalase did not perturb entry into S-phase following re-plating. Our results suggest PCB exposures could significantly impair cell (and tissue) renewal and applications of antioxidants could be an attractive redox-based therapy to treat PCB-exposed individuals. Furthermore, cell growth states quiescence vs. exponential could be a significant factor in determining cellular effects of PCBs. An additional significance of this research is our quiescent cell culture model, which we believe mimic more closely with *in vivo* growth conditions. (NIEHS P42 ES 013661)

2065 PROSTAGLANDIN INDUCED GSH EFFLUX THROUGH ABCG2.

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Glutathione (GSH) efflux signaling must contain two components: a molecule capable of inducing GSH synthesis and a transporter through which export occurs. As mediators of inflammation, prostaglandins (PGs) function in the development, progression, and resolution of inflammation. PGs can be transiently conjugated with GSH and are expelled from cells via active transport through the ATP-binding cassette transporters (ABCs). Recent evidence indicates that low concentrations of electrophilic cyclopentenone PGs (CyPG) invoke redox-based cell signaling responses including increased synthesis of intracellular GSH. Using the human derived lung cancer (A549) and head and neck cancer cells (HN4 and 1586), we show that the metabolites of PGD2 are also capable of inducing GSH efflux. In addition, we report for the first time that the ABC protein ABCG2 exports reduced GSH in response to increased GSH synthesis induced by PG treatment. A549, HN4, and 1586 cancer cells were treated with 1-6uM of various CyPGs for 48 hours. Media and cells were harvested for GSH measurements using electrochemical HPLC detection. Results indicate that extracellular GSH concentrations increase 1.5-2 fold over control in A549 and HN4 cells and by as much as 7 fold over control in 1586 when treated specifically with PGD2 metabolites. The GSH efflux is dependent on increased GSH synthesis. Based on the differential expression of ABCG2 in these cell lines, we created an inducible system for the expression of human ABCG2 in the yeast strain S. cerevisiae. GSH concentrations in the growth medium increased from undetectable in non-stimulated yeast to 0.3uM in yeast stimulated to express ABCG2. We are able to show GSH export in ABCG2 expressing yeast can be increased at least 2.5 fold over control using various stimuli. Combined, these data indicate that GSH is a newly identified substrate for ABCG2. Given the role of PGs and GSH in cell development, inflammation, and defense, ABCG2 may have an important role in signaling cross-talk between these two molecules.[Support in part by NIH HL075523]

2066 ABSENCE OF NITRIC OXIDE SYNTHASE IN EXQUISITELY PURE LIVER MITOCHONDRIA.

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The nitric oxide synthases (NOS) catalyze the conversion of *L*–arginine to *L*–citrulline plus $NO^{\cdot}$. $NO^{\cdot}$ production, near or within superoxide–rich environments, such as mitochondria, results in the generation of reactive nitrogen species (RNS). The resulting RNS, can lead to mitochondrial damage which may progress to cellular toxicity and/or death. Data, both for and against the presence of a mitochondrial NOS (mtNOS) isoform, is in the refereed literature base. Irrefutable evidence, however, for a mtNOS has not been forthcoming and conflicting reports continue to be published. In light of this controversy, we designed meticulous studies to confirm/refute the existence of mtNOS. Pure, rat liver mitochondria were obtained by differential centrifugation followed by Percoll–gradient purification. Sub–mitochondrial particles were obtained from intact mitochondria. Positive controls contained mitochondria spiked with purified, recombinant nNOS (nNOSr) prior to further processing. Following affinity chromatography and digestion with trypsin, mitochondrial protein samples were analyzed by LC-MS. The resulting data did not provide support for the presence of any NOS isoform in rat liver mitochondria whereas nNOSr-derived peptides from spiked samples (≥ 200 fmol nNOSr) were easily detected. Additionally, the protein calmodulin, required for NOS activity, could not be detected, providing further support for the absence of a NOS isoform in mitochondria. [C^{14}] *L*–arginine to [C^{14}] *L*–citrulline conversion, in the presence, or absence of NOS inhibitors, was also negative for NOS activity. Furthermore, western blot analyses using nNOS and eNOS antibodies failed to detect the presence of these two NOS isoforms in mitochondria. In conclusion, by avoiding previously published errors in methodologies and sample preparation, and by using correct controls, we confidently refute the claim that a NOS isoform exists within rat liver mitochondria. (Supported by ES011982 & RR008124 to RTM & UTEP, respectively)

2067 ROS AND CHEMOKINE RELEASE IN A549 CELLS IN RESPONSE TO FE AND SE EXPOSURE.

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Inhalation exposure to metal rich particulate matter (PM) is known to induce lung inflammation and compromise host pulmonary defense mechanisms. While production of reactive oxygen species (ROS) leading to intracellular oxidative stress is a known mechanism of action of metals, the exact molecular events that follow metal exposures that trigger the inflammatory cascade are not fully understood. In this study, the involvement of oxidative stress in the release of chemokines in response to Fe and Se exposure was evaluated using cultured human lung alveolar epithelial cells (A549). We assessed the impact of these elements on cell viability, ROS generation and IL-8 release over concentration ranges from 0.5 to 10 mM for Fe and 0.005 to 3 μM for Se for time periods up to 24 hr. Fe was not cytotoxic over the concentration range tested, but ROS measured using DCFH-DA increased over controls 1.25 and 1.5 fold at 0.005 and 0.10 mM Fe, respectively. This increase in ROS was not associated with an increased release of IL-8. Se was cytotoxic to A549 cells over a very narrow concentration range, dropping from 100% viability at 1.7 μM to 20% viability at 2.25 μM. Although no measurable change in ROS production was evident, IL-8 concentrations increased over 10-fold with increasing exposure from 1.0 to 2.75 μM Se. Results indicate that release of inflammatory chemokine by Fe and Se does not appear to be related to ROS production. Supported by NIHU56CA96203 and MD DHMH CH605CRT.

2068 POLYCHLORINATED BIPHENYL (PCB)-INDUCED OXIDATIVE STRESS MEDIATES CYTOTOXICITY IN HUMAN BREAST EPITHELIAL CELLS.

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Recent data suggested that PCBs and PCB metabolites may induce oxidative stress and cytotoxicity. To study the role of oxidative stress in PCB-induced toxicity, growth curves and clonogenic cell killing of MCF-10A human nonmalignant breast epithelial cells were measured following 5 consecutive days of media changes using serum free MEGM media with or without 3 μM PCBs in 0.1% DMSO. The results showed that Aroclor 1254, PCB153, and the 2-(4-chlorophenyl)-1,2-benzoquinone metabolite of PCB3 (4ClBQ) were cytotoxic and growth inhibitory to MCF-10A cells, with the 4ClBQ having the most pronounced effects. Using fluorescence labeling with dihydroethidine (DHE), and Mitosox, the same PCBs that were found to be cytotoxic were also found to increase steady-state levels superoxide. Moreover, toxic PCBs and their metabolites were also shown to induce 40-50% depletion of glutathione and 3-5 fold increases in manganese superoxide dismutase (MnSOD) activity. Finally, treatment with the combination of polyethylene glycol conjugated catalase (50 U/mL PEG-CAT) and superoxide dismutase (50 U/mL PEG-SOD) or the non-specific thiol antioxidant N-acetyl-cysteine (5 mM, NAC) added 1 hour after PCB exposure significantly protected the MCF-10A cells from the toxicity caused by PCBs and their metabolites. These results demonstrate that exposure to PCBs and their metabolites can induce oxidative stress and cytotoxicity in human breast epithelial cells as well as suggesting that clinically relevant thiol antioxidants such as NAC can be used to protect mammalian cells from PCB-induced cytotoxicity following exposure. (supported by NIEHS P42 ES013661)

2069 INTERACTIONS OF DINITROBENZENES WITH CALMODULIN-FREE NEURONAL NOS.

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Neuronal nitric oxide synthase (nNOS) requires calmodulin (CaM)-binding for efficient electron transfer and conversion of *L*-arginine to *L*-citrulline and nitric oxide ($NO^{\cdot}$). Thus, most studies have focused on CaM-bound nNOS. In contrast, we

have focused on CaM-free (resting state) nNOS and its interactions with dinitrobenzenes (DNBs). Turnover rates (min^{-1}) of nNOS-mediated NADPH oxidation ($NADPH_{ox}$) and acetylated cytochrome-*c* reduction (AcCyt-c_{red}) were used to estimate total electron flux and $O_2^{\cdot-}$ production. DNBs increased the rate of $NADPH_{ox}$ by CaM-free nNOS in an isomer- and concentration-dependent manner. DNB-stimulated $NADPH_{ox}$ by CaM-free nNOS, in presence of 1,4-DNB (8μM) and 1,2-DNB (40μM) equaled that of CaM-bound nNOS under similar conditions. These same concentrations were then again used in AcCyt-c_{red} assays. Rates for $NADPH_{ox}$ by CaM-free nNOS at the limit of solubility of the DNBs (160μM) were 206 ± 6 min^{-1}, 101 ± 5 min^{-1} and 32 ± 2 min^{-1} for 1,4-, 1,2- and 1,3-DNB, respectively, compared to solvent control (7 ± 1 min^{-1}). The rates of AcCyt-c_{red} by CaM-free nNOS were 136 ± 15 min^{-1}, 52 ± 11 min^{-1} and 72 ± 20 min^{-1} in presence of 1,4-, 1,2- and 1,3-DNB, respectively. Thus, 1,4-DNB was the most potent DNB isomer in stimulating $O_2^{\cdot-}$ production by CaM-free nNOS. Using the $NADPH_{ox}$ assay, the nNOS reductase construct (aa 725-1729) behaved in a manner similar to holo-nNOS and NADPH-cytochrome P-450 reductase in the presence of DNBs. Collectively, these results indicate that CaM-free nNOS generates ROS at environmentally-relevant levels of DNBs. Considering the absolute amount of constitutive NOS expressed in organisms, chronic exposure to low levels of DNBs leads to continuous oxidative stress (increased NADPH oxidation, generation of ROS, depletion of cellular energy stores). These effects may act to accelerate the development of chronic degenerative illnesses.
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2070 MITOCHONDRIAL PEROXIREDOXIN III FUNCTION IN PROTECTION AGAINST OXIDATIVE STRESS.

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2,3,5-tris(Glutathion-S-yl)hydroquinone (TGHQ), a reactive metabolite of hydroquinone, induces necrotic cell death in renal proximal tubule epithelial cells (LLC-PK1) by generating reactive oxygen species (ROS). However, the mechanisms by which TGHQ induces necrosis are unclear. Peroxiredoxin III (PrxIII), located exclusively in the mitochondrion, is a member of the antioxidant family of thioredoxin-dependent peroxidases. It is thought to contribute to the primary antioxidant defense of the mitochondrial respiratory chain. We have previously observed that in LLC-PK1 cells, PrxIII is present in both the monomeric and dimeric forms, the latter presumably arising via the endogenous generation of reactive oxygen species (ROS). Following exposure to TGHQ, PrxIII is further oxidized to the cysteine sulfinic acid. Two-dimensional gel electrophoresis followed by MALDI-TOF analysis revealed that TGHQ actually induced two acidic shifts in PrxIII, with both pI shifted spots representing oxidized forms of PrxIII. Furthermore exposure to TGHQ resulted in the majority of PrxIII being converted into higher molecular weight complexes, in a concentration- and time-dependent manner, analogous to that reported for PrXII. To determine the role of PrxIII in protection against TGHQ-mediated mitochondrial oxidative stress, we used LLC-PK1 cells stably expressing a dominant negative version of PrxIII, in which two active cysteine residues were substituted to serine residues (C108S, C229S). Dominant negative PrxIII overexpression, but not wild-type PrxIII, significantly attenuated TGHQ-induced cytotoxicity. The data suggest that PrxIII blocks TGHQ-induced cellular toxicity presumably by reducing TGHQ-mediated mitochondrial ROS generation. Changes in the pattern of the redox state of PrxIII might be a useful marker of ROS-mediated kidney disease. (RO1 DK59491, P30 ES 06694)

2071 HYPEROXIA ENHANCES LPS-INDUCED HMGB1 RELEASE FROM ALVEOLAR MACROPHAGES AND EPITHELIAL CELLS.

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Eexposure to supraphysiological concentrations of oxygen (hyperoxia) during mechanical ventilation is associated with pulmonary toxicity. Hyperoxia-induced acute lung injury is associated with an influx of inflammatory cells, enhanced cytokine production and death of pulmonary epithelial and endothelial cells. HMGB1, a 30kd nonhistone nuclear protein, has been shown to mediate the development and progression of acute lung injury-induced by endotoxin. Alveolar macrophages (AM), the resident phagocytes in the alveolar space, play a pivotal role in the innate immune response in the lung. To determine the effects of hyperoxia on endotoxin-mediated proinflammatory responses, Raw 264.7 cells, a macrophage-like cell line, were exposed to LPS (1μg/ml) and hyperoxia (95% O2 for 24 h) either alone or in combination. Hyperoxia enhanced LPS-induced release of HMGB1 from RAW cells compared to those treated with LPS alone. We further examined whether the duration of LPS treatment would affect HMGB1 release. RAW cells were treated with LPS for 3 h followed by 24 h hyperoxic exposure. Interestingly simultaneous treatment of LPS and hyperoxia for 24 h showed an enhanced release of HMGB1 compared to 3 h LPS treatment followed by hyperoxia. A similar increase in HMGB1 release was also observed in cells treated with LPS alone suggesting that the prolonged presence of LPS is important for the sustained release of HMGB1 from macrophages. A549 cells, a transformed human cell line with characteristics of alveolar epithelial cells were also simultaneously exposed to hyperoxia (95% O2) and LPS (1μg/ml) for 24 h. Indeed, the release of HMGB1 induced by LPS was enhanced by hyperoxia even in epithelial cells. Our current results indicate that both alveolar macrophages and epithelial cells may contribute to recruitment of phagocytes that further amplify the inflammatory response.

2072 MECHANISTIC STUDY OF NRF2 ACTIVATION BY ARSENIC.

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Arsenic is a widely-spread environmental toxicant and has been classified as a human carcinogen. Reactive oxygen species (ROS) is one of the multiple mediators of arsenic-induced toxicity. Nrf2-dependent antioxidant response is the main defensive mechanism against ROS in eukaryotic cells and has been recently shown to be activated upon arsenic treatment. Elucidating the molecular mechanism of Nrf2 activation by arsenic is important for understanding the protective role of Nrf2 against arsenic-mediated toxicity and carcinogenicity. Under normal cellular redox conditions, Nrf2 is negatively regulated by Keap1 through ubiquitination and subsequent proteasomal degradation. In response to tert-butylhydroquinone (tBHQ) or sulforaphane, Nrf2 escapes ubiquitination and degradation in a manner that involves cysteine 151 of Keap1, suggesting a role of Keap1 as a redox sensor. Here we show that arsenic-mediated activation of Nrf2 is independent of cysteine 151 on Keap1, indicating the existence of a distinct mechanism of Nrf2 activation. We hypothesize that arsenic alters the dynamic assembly and disassembly of the Keap1 ubiquitin ligase complex, resulting in enhanced Keap1 self-ubiquitination and decreased Keap1 stability.
[NIEHS 1RO1ES015010-01]

2073 MODERATE CONCENTRATIONS OF HYDROGEN PEROXIDE RESCUE HYPEROXIA-INDUCED MACROPHAGE DYSFUNCTION.

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Exposure to prolonged hyperoxia as seen in patients on mechanical ventilation is widely recognized to be associated with the generation of excessive superoxide. Our previously studies and others have reported that elevated levels of superoxide leads to compromised ability to phagocytize bacteria, predisposing the patient population to ventillator-associated pneumonia. On the other hand, hydrogen peroxide (H2O2) plays an important role in host defense and can act as a second messenger in the activation of various signaling pathways, including those involved in antioxidant defenses, removal of damaged cells and host repairing mechanisms. In this study, we tested the effects of H2O2 on hyperoxia-induced impairment of macrophage function. Hyperoxic RAW 264.7 cells were treated with concentrations of H2O2 from 10 μM to 1mM for one hour. Interestingly, moderate concentrations of H2O2 significantly reversed hyperoxia-induced cell cycle arrest and hyperoxia-inhibited macrophage migration. However, at a high dose of 750μM, H2O2 exhibited a toxic effect on macrophage function. Surprisingly, prolonged exposure to hyperoxia for 24 or 48h, along with a high dose of 750μM H2O2 appeared to be less toxic compared to those treated with 750μM H2O2 alone, determined by cell morphology. These findings are particularly important because the incidence for ventilator-associated pneumonia remains unacceptably high. We are currenly investigating mechanisms by which H2O2 modulate hyperoxia-induced macrophage dysfunction.

2074 THE IKKβ CROSSTALKS WITH THE TGFβ PATHWAYS IN ARSENIC TOXICITY.

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Arsenic compounds are environmental hazards, causing diverse health problems in exposed humans and animals. Recently, arsenic has also been used to treat acute promyelocytic leukemia that significantly improved survival of the patients. We

have previously established that IκB kinaseβ (IKKβ) is crucial for antagonizing arsenic toxicity. IKKβ, acting through activation of nuclear factor κB (NF-κB) and up-regulation of anti-oxidant genes, can suppress reactive oxygen species (ROS) accumulation and cell death induced by arsenic. Thus, the IKKβ (-/-) fibroblasts are more susceptible than their wild type counterparts to arsenic-induced death. Moreover, the IKKβ (-/-) cells grow much slower, suggesting altered growth controlling system in the null cells. The transforming growth factor β (TGFβ) is known to inhibit fibroblast growth via activation of the Smad transcription factors. We hypothesize that the TGFβ activity is disturbed in the IKKβ (-/-) cells partly responsible for sensitivity to arsenic toxicity. We performed real-time PCR on RNAs isolated from wild type and IKKβ (-/-) fibroblasts and examined the expression of TGFβs and their receptors. We found 2-7-fold increase of TGFβ 2/3 and their receptors TGFβ-receptor I/II expression in IKKβ (-/-) over wild type fibroblasts. The elevated TGFβ signaling in the IKKβ (-/-) cells appeared to contribute to arsenic toxicity, because inhibition of TGFβ signaling by pre-treatment the IKKβ (-/-) fibroblasts with a TGFβ-receptor inhibitor, SB505124, and by overexpression of the inhibitory Smad7, could reduce arsenic-induced cell death. Cell death was measured by observing the condensed nuclei as an indicator of apoptosis and by the relative levels of lactate dehydrogenase release. Conversely, pre-treating the wild type cells with TGFβ caused about 10-fold increase of cell death in response to arsenic. We suggest that IKKβ may negatively regulate the TGFβ pathway and that such crosstalk contributes to cell protection against arsenic toxicity.

2075 INVESTIGATION OF THE EFFECT OF OXIDATIVE STRESS ON THE PROTEIN STABILITY OF THE INSULIN TRANSCRIPTION FACTOR, MAFA.

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Type II diabetes is characterized by chronic hyperglycemia, which is caused in part by decreased insulin production. Previous research by Robertson and associates using a mammalian pancreatic cell line (HIT-T15) has shown that levels of MafA, a transcription factor required for insulin production, are decreased after chronic culture in high glucose. Degradation of this protein appears to be triggered by reactive oxygen species as evidenced by the fact that co-culture with the antioxidant N-acetyl-cysteine has been shown to prevent the degradation of MafA protein. Although oxidative stress is hypothesized to cause the degradation of MafA, little is known about the role of antioxidant enzymes in determining MafA stability at the molecular level. Saccharomyces cerevisiae, a model system that has been used to characterize the role of protein degradation in a variety of human diseases, is a useful model in which to study the effect of reactive oxygen species on MafA protein stability. An expression vector was designed to express murine MafA in yeast. Expression of the mouse protein in yeast was verified by western blot. This functional model system will be used in subsequent experiments to examine the role of endogenous anti-oxidant enzymes including gamma glutamylcysteine synthetase (Gsh1), glutathione synthetase (Gsh2), glutathione peroxidase (Gpx1 and Gpx2), superoxide dismutase (Sod 1 and Sod 2), and catalase (Ctt1) in modulating MafA stability in the presence of reactive oxygen species.

2076 EFFECTS OF SODIUM ARSENITE ON ROS PRODUCTION AND CELL PROLIFERATION MEDIATED BY C-MYC AND NF-κB INDUCTION IN MCF-7 CELLS.

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Arsenic (As) exposure has been linked to cancer of the urinary bladder, lung, liver, and skin among other potential target sites. As metabolism plays a relevant role in As toxicity, since it generates As-methylated species, therefore depleting methyl donor pools, which may result in DNA hypomethylation and genomic instability. In several cell types, As exposure increases reactive oxygen species (ROS) production which are involved in DNA oxidative damage, as well as activation of transcription factors and expression of genes that regulate proliferation and apoptotic processes.

Our aim was to evaluate the effects of acute As exposure on ROS generation, NF-κB activation, c-Myc levels and cell cycle profile in the human breast cell line MCF-7. Treated cells showed increased growth rate, as measured by trypan blue exclusion test and increased cell recruitment in S-phase of cell cycle as evaluated by FACS. There was an increased ROS production as measured by the DCFH-DA method and ROS-mediated DNA damage as measured by the presence of 8-OH-dG DNA-adducts. Treated cells also showed increased levels of c-Myc and NF-κB proteins and phosphorilated IκB protein on a concentration-dependent fashion. These effects suggest that the ROS-mediated induction of genes regulating cell proliferation, such as c-myc and NF-κB, plays an important role in the proliferation of cells that have suffered genomic instability processes produced by hypomethylation and by ROS-mediated DNA damage.

2077 OXIDATIVE POTENTIAL AND CELLULAR EFFECTS INDUCED BY PM10 OBTAINED IN MEXICO CITY AND AT A RECEPTOR SITE.

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Current working hypothesis on the mechanisms mediating particulate matter (PM) toxicity resides on its capacity to induce oxidative stress on cells. PM metals and organics have the potential to cause it. However, further understanding of PM composition-cellular effects is needed. As part of the MILAGRO campaign we sampled PM10 in Northern Mexico City and in a receptor site outside the city (NE) in order to compare and link composition, oxidative potential and ventilatory patterns, with cellular effects. PM was sampled daily during March 2006 on cellulose membranes using High Vol samplers. Samples physically removed from the membranes were pooled accordingly to 5 identified ventilatory patterns. Metals and organics paramagnetic resonance signal were measured by Electron Paramagnetic Resonance (EPR). PM oxidative potential was measured by EPR using DMPO. Carbon (TOR), ions(IC) and elemental (ICP-MS & PIXE) PM content was analyzed in all samples. DNA damage was assessed electrophoretically using "naked" DNA and cell membrane disruption by hemolytic assay, using 5-160 μg/mL of PM10. Principal component analysis was performed on 46 variables in order to explore groups of PM and biological factors based in their structure correlation. The 46 set of variables was reduced to 7 components (C1..C7). Only C1, C2 and C6 correlated with biological relevant effects. Hemolysis was associated with Si, Al, P, S & OC (C1); oxidative potential with Cu, Zn & Ba (C2); and DNA degradation with V & Cr (C6). C6 showed a positive tendency with ventilatory patterns; as the only indicator of pollutants from the city reaching the receptor site. Our results indicate that PM composition determines different cell outputs. However, they did not correlate with the PM components identified by EPR as having oxidative potential. Supported by MILAGRO-CAM and CONACyT 43138-M.

2078 DNA DAMAGE BY A TWO-ELECTRON OXIDATION (EPOXIDATION) MECHANISM.

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Oxidative damage to nucleobases can impact not only DNA, potentially leading to mutations, cancer, aging, and neurological disorders, but also cell signaling and energy transfer. The oxidative stress that gives rise to the reactive oxygen species (ROS) responsible for such damage can come from many sources both endogenous and exogenous, and the ROS can act by different mechanisms. Hence we sought to determine the extent to which specific mechanisms give rise to distinct oxidation products, to aid in linking outcomes to particular exposures. We used a model two-electron oxidant, dimethyldioxyrane, that mimics epoxidation by inserting an oxygen atom across double and aromatic bonds, to oxidize the purine nucleobases *in vitro*. Products were identified by a combination of liquid chromatography-electrospray mass spectrometry, proton and multidimensional nuclear magnetic resonance, and UV spectroscopy. Product profiles were determined by high pressure liquid chromatography. Guanine, the most easily oxidized of the nucleobases, yielded almost exclusively a novel oxidation product, 5-carboxamido-5-formamido-2-iminohydantoin. The iminohydantoin product was stable under the reaction and work-up conditions. Two-electron oxidation of adenine proceeded more slowly and gave mainly adenine 1-oxide, a known carcinogen, adenine 3-oxide, which underwent further oxidation, and possibly small amounts of adenine 7-oxide and adenine 9-oxide. 8-Oxoguanine or 8-oxoadenine were not seen in these reactions, although both are formed by reaction with hydroxyl radicals and the former is a widely-used biomarker for oxidative stress. 5-Carboxamido-5-formamido-2-iminohydantoin may thus be able to serve as a marker for two-electron oxidation processes *in vivo*.

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2079 OXIDATIVE DNA DAMAGE IN NCI-H292 CELLS EXPOSED TO CIGARETTE SMOKE AT THE AIR-LIQUID INTERFACE.

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Cigarette smoke is a complex dynamic mixture of more than 4,000 chemicals distributed between the particulate and vapour phases. It is widely acknowledged that cigarette smoke is capable of causing oxidative damage, either directly or through generation of reactive oxygen species. Oxidative damage to biomolecules such as lipids, proteins and DNA have been implicated in many chronic and degenerative human diseases as well as aging. More importantly, the link between oxidative DNA damage and carcinogenesis is stronger than ever. Therefore, it's important that we can reproducibly measure and quantify the amount of oxidative DNA damage following exposure.

To investigate the potential oxidative effects of cigarette smoke on DNA, we employed the modified in vitro Comet assay using the lesion specific enzyme FormamidoPyrimidine Glycosylase (FPG), in combination with a whole smoke exposure system. This differs from traditional toxicological assessment using cigarette smoke condensate, as it combines both particulate and vapour phases of mainstream cigarette smoke in one exposure system.

Results obtained, demonstrate that NCI-H292 cells exposed to 30 minutes diluted mainstream cigarette smoke at the air-liquid interface generate excessive strand breaks, alkali labile sites and oxidative lesions, all of which can be measured using the Comet assay. Oxidative lesions, post exposure, are confounded by high levels of strand breaks and are indistinguishable. By allowing a period of recovery (20-hours) strand breaks repair thus leaving the oxidative lesions, which can be measured and semi-quantified.

It is believed that the whole smoke exposure system combined with the modified in vitro Comet assay may give valuable information when investigating the effects of whole smoke and its individual constituents. Moreover, the Comet assay is a sensitive tool for assessment of oxidative lesions using the lesion specific enzyme FPG.

2080 UNCONJUGATED BILIRUBIN CAUSES CHANGES IN INTRACELLULAR REDOX STATUS, CHANGES TO THE DISULFIDE PROTEOME, AND UPREGULATION OF SEVERAL GENES INVOLVED IN ER STRESS.

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Deposition of unconjugated bilirubin (UCB) in the central nervous system causes kernicterus in some severely jaundiced neonates. The molecular mechanisms that contribute to UCB neurotoxicity remain unknown; however, we and others have shown that μM concentrations of UCB cause oxidative stress. The objective of the current study was to employ novel, redox sensitive, green fluorescent proteins as well as 2D redox gel electrophoresis to determine the redox status of murine hepatoma (Hepa 1c1c7) cells exposed to 70 nM or 50 μM UCB in real time. UCB (50 μM) caused initial oxidation of the intracellular compartment by 30 min that was partially reversed at 2 h. By 3 - 6 h post-treatment, irreversible oxidation of the intracellular compartment was observed, concomitant with appearance of early markers (caspase-3) for apoptosis. In contrast, 70 nM UCB showed a pronounced increase in the reduction of the intracellular compartment between 3 and 6 h post treatment. Two dimensional redox gel electrophoresis experiments indicated that several proteins containing reactive cysteine thiols were oxidized in cytosol of Hepa 1c1c7 cells treated with 50 μM UCB whereas some protein disulfide bonds were reduced in the endoplasmic reticulum (ER). Microarray experiments were performed to compare changes in gene expression following treatment with 50 μM and 70 nM concentrations of UCB. A number of genes involved in the response to ER stress were found to be upregulated, suggesting that ER stress may play a role in UCB toxicity, in addition to the apoptosis that results from mitochondrial signaling that we previously described.

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2081 MULTI-PARAMETER ANALYSIS OF OXIDATIVE STRESS IN INDIVIDUAL CELLS USING HIGH CONTENT IMAGING AND QUANTITATIVE ANALYSIS.

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Oxidative stress is a consequence of aerobic respiration, and increased levels of reactive oxygen species (ROS) result in damage to DNA, proteins, lipid peroxidation and metabolic dysfunction. Accumulation of damage from oxidative stress has been implicated in aging, cancer, and other diseases. Cells combat oxidative stress and damage through induction of enzymes, such as superoxide dismutase (SOD) and catalase to reduce levels of superoxide and hydrogen peroxide. DNA and protein repair enzymes are induced in response to oxidative DNA damage. To study the effects of oxidative stress in an individual cell, ideally one would want to measure production of the toxic oxygen species, the damage that occurs, as well as the response of the cell to repair the damage over time. For this study, a high content cell-based imaging approach was used to measure superoxide production, mitochondrial function, DNA damage, and cellular repair response. Cells were treated with a variety of stress reagents, including etoposide, a known double-strand DNA-damaging agent and rotenone, a superoxide generating drug and monitored for cellular changes. Oxidative stress and resulting damage was measured using cytochrome C, heme oxygenase, mitochondrial dyes, superoxide production, MnSOD, phosphohistone 2AX, and 8-oxoguanine DNA lesions as targets. Compounds were ranked accordingly for potency in the ability to induce oxidative stress, DNA damage, or both oxidative stress and DNA damage. This study demonstrates that the use of high content cell-based imaging analysis enables measurement of multiple targets within the same cell to obtain a correlative understanding of both individual cellular responses and those of the total cell population. This approach will be a powerful tool in further understanding the biology of oxidative stress, as well as in lead compound development and oxidative toxicology.

2082 APPLICATION OF CONFOCAL AUTOFLUORESCENCE SPECTROSCOPY FOR ANALYSIS OF NADH STATUS IN THE HEPG2 AND THE C6 GLIOMA CELLS AND ITS CHANGE UPON EXPOSURE TO CD.

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NADH and FAD are important cofactors in cells that help catalyze oxidation-reduction reactions. Using the confocal spectroscopy, the amount of NADH and FAD can be detected due to their intrinsic autofluorescence properties. The ratio of FAD/NADH serves as a marker that reflects cellular redox state. Together with the time-resolved confocal fluorescence spectroscopy, which analyzed the rate of NADH fluorescence decay, the level of free/bound NADH could also be determined. In the present study, these methods were applied to study NADH changes in the HepG2 and the C6 glioma cells. Under resting condition, FAD/NADH in the HepG2 was similar to that in the C6 glioma cells. Both cells showed a gradual increase in FAD/NADH over a 3 hr exposure to Cd. The C6 glioma cells, however, was less tolerant to Cd in that at 0.1 mM, a 3 hr exposure already caused a significant (30%) increase FAD/NADH in these cells while similar changes was not evidenced in the HepG2 cells unless Cd reached 0.6 mM. The change in FAD/NADH was also associated with an increase in free NADH in both cell lines. NADH is an important regulatory substrate to lower enzymatic (e.g. pyruvate dehydrogenase, phosphofructose kinase, ketoglutarate dehydrogenase) activity along the metabolic pathway leading to a slow down in energy production. Thus, results of the present study suggested that comparing to the HepG2 cells, one of the reasons that C6 glioma cells is more susceptibility to Cd injuries may be due to its ability to release NADH from its bound form thus, reducing energy production and lead to subsequent cell death.

2083 HUMAN *IN VITRO* HEMOLYSIS MODEL TO ASSESS DRUG-INDUCED HEMOLYTIC EFFECTS.

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A human in vitro model to investigate drug induced hemolytic effects incorporates a precision-cut liver slice co-cultured with blood-medium for up to 72 hr. The value of using a liver slice and whole blood is by the presence of myeloperoxidase in kupffer cells and leukocytes, which may contribute to the metabolism of the compound. Liver kupffer cells also participate in heme homeostasis and turnover. Methimazole (MMI) causes a time-dependent and concentration-dependent increase in hemolysis. Hemoglobin release is significantly increased with 2-3 mM MMI at 24 hr which progresses with time, and at 1 mM MMI by 48 hr. In the absence of a liver slice, hemolysis is ≤10% at 72 hr with MMI, which is equivalent to control values. Blood GSH levels decline significantly by MMI within 24 hr, prior to hemolysis. Blood ATP levels decline significantly in the presence of a liver slice, while in the absence of a liver slice, blood ATP levels decline but recover and are equal to control values by 72 hr. Activation of liver heme-oxygenase 1 (HO-1), which is involved with heme homeostasis and degradation, is positively correlated with he-

molysis. An up-regulation of HO-1 gene expression is evident at 24 hr with 3 mM MMI, at ≥1 mM at 48 hr and at ≥0.5 mM at 72 hr. Hepcidin, a negative regulator of iron release from cells, is down-regulated by MMI 0.5-3 mM at 48 hr. IL-8 expression, indicative of endothelial cell activation, likely by hemoglobin, is up-regulated by MMI ≥ 2 mM at 48 hr. This study demonstrates that the liver slice-whole blood hemolysis model provides mechanistic insight into compounds that generate oxidative stress leading to blood GSH depletion, and changes in heme pathway gene expression and hemolysis.

2084 A NEW 405 NM LASER EXCITABLE FLUORESCENT PROBE OF INTRACELLULAR THIOLS FOR FIXED END-POINT IMAGE BASED ASSAYS.

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Reduced glutathione represents the majority of intracellular free thiols in mammalian cells. Among the many important physiological and pathological aspects involved, glutathione plays a central role in protecting mammalian cells against damage incurred by free radicals, oxidants, and electrophiles. Currently available fluorescent probes for detecting intracellular thiols in live cells share drawbacks ranging from low fluorescent intensity, species-dependence of labeling, to a requirement of UV excitation. We have developed a new fluorescent cell-permeant thiol reactive probe with the following property: (1) Maximal excitation at 410 nm and maximal emission at 510 nm; (2) Labeled cells can be detected with standard fluorescence microscopy with a filter set for Hoechst 33342 (Ex/Em = 350 nm/461 nm) ; (3) Labeled cells can be detected with a 405 nm laser equipped flow cytometer or laser scanner; (4) Signal intensity and cellular staining pattern are well retained after formaldehyde fixation and 0.5% (v/v) Triton X-100 extraction. In formaldehyde fixed cells viewed with a fluorescence microscope with a standard filter set for Hoechst 33342, the signal intensity of the new probe is estimated to be at least 10 times brighter than monochlorobimane (mBCl, glutathione conjugate, Ex/Em = 394 nm/490 nm) and at least 2 times brighter than 7-amino-4-chloromethylcourmarine (CMAC, Ex/Em = 353 nm/466 nm, not usable with 405 nm laser based instruments). By using automated microscopy and image analysis, responses of cultured human or murine cells of liver or kidney origin to treatments with agents that affect the intracellular glutathione level, such as buthionine sulfoximine (BSO), diethyl maleate (DEM), quinone toxicants including benzoquinone, were readily quantifiable by labeling with this new probe.

2085 ROLE OF METALLOTHIONEIN IN HEPATIC TOXICITY CAUSED BY VANADIUM COMPOUND IN MICE.

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We have examined the toxicity of the ammonium metavanadate (AMV) which is pentavalent vanadium compound. It was clarified that hepatic toxicity of AMV was enhanced by depression of reduced glutathione (GSH) in blood plasma. We have also shown that the AMV induced hepatic metallothionein (MT), and that the induction by AMV was partly dependent on the production of inflammatory cytokine, interleukin-6 (IL-6). MT, as well as GSH, has SH residue, and is concerned in metabolism and detoxication of heavy metals. The experiment was carried out using MT-I/II null mice in order to clarify the role of MT in the vanadium toxicity. MT-I/II null mice and wild-type mice (OLA129/C57BL6) were received a single subcutaneous administration of AMV (0.1 or 0.3 mmol/kg), and the animals were sacrificed 24 h after the administration. In wild-type mice, activities of AST, ALT and ALP increased at 0.3 mmol/kg. Contents of BUN and Cre were increased at same dosage. These results showed that dosage of 0.3 mmol/kg caused the damage in liver and kidney. On the other hand, when MT-I/II null mice were treated with 0.3 mmol/kg AMV, hepatic and renal injuries were significantly increased compared with the wild-type mice. In histological analysis, swelling and necroses of the hepatocytes, and necrosis of the renal tubules and urinary casts in the cortical area were observed more frequently in the MT-I/II null mice. There were no difference in the GSH concentrations in liver and kidney, between MT-I/II null mice and wild-type mice. Total vanadium contents in organs of animals were measured using ICP-MS. The vanadium concentration in liver and kidney of the MT-I/II null mice were higher than those of wild-type mice. HPLC/ICP-MS analysis on the distribution of the metals in hepatic cytosol of animals treated with AMV revealed that the major metal bound to MT was not vanadium, but zinc. Though MT was not combined with the vanadium, it was shown that MT is related to strength of the toxicity of the vanadium.

2086 ULTRASTRUCTURAL NUCLEAR CHANGES IN LYMPHOCYTES AFTER INHALED VANADIUM EXPOSURE.

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Vanadium (V) is part of the TSP and through this route enters into the respiratory system. The main source of this element in the air is the combustion of oil products. In vitro vanadium induces aneuploidogenic, mutagenic and clastogenic actions, and also increases micronuclei frequency, as well as interference in the cell cycle by its effect on cytoskeleton proteins. On the other hand antiapoptotic and proliferative effects have been found.
As a consequence of these contradictory actions, we decided to evaluate nuclear ultrastructural changes in lymphocytes of mice exposed to inhaled vanadium, since one criterion for the identification of neoplastic cells is its nuclear architecture.
Male mice were exposed to V2O5 (0.02M) twice a week for three months, and at the same time controls were exposed to distilled water with the same schedule. Animals were sacrificed by lethal IP injection of pentobarbital, perfused with glutharaldehyde and paraformaldehyde 2%. Spleens were removed and processed for ultrastructural analysis. From each mouse, fifteen fields were evaluated
Results: Changes observed were chromatin reorganization, invaginations, evaginations, swollen perinuclear cysterna, increased nuclear pores and pseudoinclusions. These changes were strikingly higher in exposed mice compared with controls. Also ultrastructural changes increased along exposure time.
Conclusions: The ultrastructural modifications found are characteristic of neoplastic cells, which might be related with the interaction of V with nuclear cytoskeleton
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2087 HIF-1α AND COX-2 MEDIATE SYNERGISTIC RELEASE OF ANGIOGENIC FACTORS FROM HUMAN LUNG FIBROBLASTS IN RESPONSE TO NICKEL AND MICROBIAL STIMULI.

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We have previously shown that NiSO4 (Ni) and the toll-like receptor 2 agonist, macrophage-activating lipopeptide (MALP-2) synergistically interact to amplify the release of the pro-angiogenic chemokine CXCL8 from human lung fibroblasts (HLF) via a COX-2-dependent pathway. Here, we sought to determine if additional angiogenic mediators were differentially modulated by combined Ni and MALP-2 exposure using an Angiogenesis pathway-specific PCR Array covering 84 genes with known function in neo-vascularization. HLF were treated with NiSO4 (200 μM), MALP-2 (600 pM) or the two stimuli together for 48 h. Following treatment, cells were harvested for RNA and conditioned medium was collected for ELISA analysis. PCR Array results indicated that in addition to CXCL8, Ni synergistically enhances MALP-2-induced gene expression of CXCL1, among others (CXCL3, 5, and 6). Additionally, Ni-induced expression of VEGF was further enhanced by MALP-2. ELISA analysis of conditioned medium validated the array findings for Ni/MALP-2 interactive effects on CXCL1, CXCL5, and VEGF. Ni and MALP-2 co-treatment similarly induced COX-2 gene and protein expression. Transfection of HLF with 50 nM COX-2 siRNA reduced both CXCL1 and VEGF release by about 60% following Ni and MALP-2 exposure, indicating a COX-2-mediated pathway similar to what was observed previously with CXCL8. Exposure of HLF to Ni and MALP-2 further stabilized expression of HIF-1α, a hypoxia-inducible transcription factor that promotes neo-angiogenesis, compared with Ni alone. HIF-1α siRNA (50 nM) prevented the induction of COX-2 and significantly attenuated Ni and MALP-2-induced CXCL1, CXCL8 and VEGF release by approximately 80%. The current findings indicate that Ni and MALP-2 interact via COX-2 and HIF-1α-dependent signaling pathways to upregulate the expression and release of multiple HLF-derived factors that stimulate neo-vascularization during combined exposure to chemical and microbial stress. Supported by RO1-ES011986 and 1F32ES015966.

2088 OVEREXPRESSION OF F-BOX PROTEIN, HRT3 OR YLR224W, CONFERS RESISTANCE TO METHYLMERCURY IN YEAST CELLS.

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Methylmercury (MeHg), the predominant form of organic mercury present in the environment, is a toxic compound producing severe disorders in the human central nervous system. However, mechanisms of MeHg toxicity and cellular protective mechanisms against such toxicity remain poorly understood. We have previously analyzed the mechanism of acquisition of resistance to MeHg that involves the

overexpression of Cdc34, a ubiquitin-conjugating enzyme, and we have shown that enhancement of cellular proteolysis by the ubiquitin-proteasome system (UP system) helps to protect cells against the toxic effects of MeHg. Thus, we postulated that some protein(s) involved in MeHg toxicity might be included among the proteins whose degradation is enhanced by the enhanced activity of the UP system. Identification of such a protein(s) would provide an important clue to the mechanism of expression of the toxicity of MeHg. F-box proteins have their own respective substrate-specificities, playing important roles in the selection of proteins that are degraded by the UP system. In order to identify the protein(s) involved in MeHg toxicity that is degraded by the UP system, we need to identify the F-box protein(s) that is involved in the recognition of this protein(s). In this study, we searched for F-box proteins that might be related to the mechanism that protects Saccharomyces cerevisiae against the toxic effects of MeHg. We found that overexpression of Hrt3 or of Ylr224w rendered yeast cells strongly resistant to MeHg. Yeast cells that overexpressed Hrt3 or Ylr224w were barely resistant to MeHg in the presence of a proteasome inhibitor. Our results suggest the existence of some protein(s) that enhances the toxicity of MeHg in yeast cells and, also, that overexpression of Hrt3 or Ylr224w can confer resistance to MeHg by enhancing the polyubiquitination of this protein(s) and its degradation in proteasomes.

2089 THE ROLE OF MRP2 AND GLUT2 TRANSPORTERS IN THE METABOLISM OF ARSENITE BY PRIMARY HUMAN HEPATOCYTES.

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The metabolic fate of arsenic (As) in human cells is determined by a number of factors, including the activity of As(+3 oxidation state) methyltransferase (AS3MT). AS3MT catalyzes a sequence of reactions that convert inorganic As (iAs) to methyl-As (MAs) and dimethyl-As (DMAs) metabolites. We have previously shown that although AS3MT expression is essential for iAs methylation, other factors may also play important roles in determining the overall capacity of human cells to methylate iAs. The present work examined relationship between the expression of potential As transporters (AQP9, GLUT2, P-gp, MRP1 and MRP2) and the production and cellular retention of iAs, MAs and DMAs in primary cultures of human hepatocytes exposed for 24 hours to subtoxic concentrations of arsenite. Our results show that the retention of iAs and MAs by hepatocytes exposed to low concentrations of arsenite correlates negatively with MRP2 expression. A positive correlation was found between MRP2 expression and DMAs production, specifically, the concentration of DMAs in culture media. After exposures to high concentrations of arsenite which almost completely inhibited AS3MT-catalyzed methylation, a positive correlation was found between the expression of GLUT2 and cellular retention of iAs and MAs. AQP9 or P-gp expression had no effect on the production or distribution of iAs, MAs or DMAs, regardless of the exposure level. Hepatocytes used in this study did not contain detectable amounts of MRP1. These data suggest that MRP2 plays an important role in the efflux of DMAs, thus, regulating kinetics of the methylation reactions and accumulation of the intermediary metabolites by human hepatocytes. The membrane transport of iAs by the high-capacity GLUT2 transporters is not a rate limiting step for the metabolism of arsenite at low exposure level, but may play a key role in accumulation of iAs after acute exposures.

2090 DNA DAMAGE IN HUMAN LYMPHOCYTES IN VITRO EXPOSED TO COMBINED METALS; ARSENIC, CADMIUM, COBALT AND CHROMIUM.

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Human are exposed daily to low level of environmental and occupational metals. Therefore, results of the toxicity tests which conducted on single-chemical at high level exposure are difficult to extrapolate to the risk assessment of the chemical mixtures in these environments. In this study, we focused on four metals (arsenic, cadmium, cobalt and chromium) which are recognized as carcinogens or co-carcinogens, and evaluated whether the DNA damage in human lymphocytes *in vitro* exposed to binary mixture of metals at low level was additive, synergistic or antagonistic. Human primary lymphocytes were extracted from four healthy donors. Single-strand DNA breakage induced by metals in lymphocytes was evaluated by comet assay. We confirmed that dose-related significant increase in the DNA damage score (%DNA in tail) after one hour single-metal exposure. The minimum dose of metal which significantly increased compared to non treated sample were determined as lowest observed adverse effect level (LOAEL) of DNA damage. The individual LOAEL of DNA damage with single-meal were decided by each donor. The ranges of LOAEL were As: 5-10μM, Cd: 250-500μM, Co: 25-250μM, Cr: 5-10μM. In binary mixture of metals As-Cd, As-Co, As-Cr, Cd-Co, Cd-Cr and Co-Cr, metals were mixed at a half LOAEL. DNA damage in binary mixture was evaluated by comet assay as well as in single-exposure. There were no significant differences of DNA damage between single-exposure and binary mixture of metals on any metal combinations. Therefore, deviations from additivity were not observed. Our results support the hypothesis that individual exposed to mixtures of chemicals at low non-toxic dose is additive or less than additive.

2091 HETEROLOGOUS EXPRESSION SYSTEM IN YEAST REVEALED STRUCTURE-FUNCTION RELATIONSHIP IN METAL TRANSCRIPTION FACTOR, MTF-1.

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Organisms have to adapt to fluctuations in metal concentrations in their environment. Non-essential metals are exported from the cell or sequestered in subcellular compartments. In contrast, essential metals are absorbed, stored and distributed within the cell, however, in excess essential metals can be harmful. Metallothioneins (MTs) play important roles in metal homeostasis and detoxification. The metal-inducible transcription of MT is controlled by the interaction between MREs (metal responsive elements) in their promoters, and the MRE-binding transcription factor, MTF-1. It has been proposed that metal-responsive transcriptional activity of MTF-1 is controlled by its phosphorylation status. This model is supported by the observations that MTF-1 is phosphorylated in vivo and that activators and inhibitors of signal transduction cascades affect MT transcription. A heterologous yeast expression system was used to measure the transcriptional activity of MTF-1. In this system, the expression of MTF-1 is regulated by the GAL1 promoter in a strain of yeast in which the promoter region of ADE2 gene has been replaced with minimal CYC1 promoter and multiple copies of MRE. We confirmed that the number of MREs and level of MTF-1 expression are responsible for reporter gene expression. This system was used to screen randomly mutagenized MTF-1. Forty-five loci, which are primarily located within the zinc-finger domain, produced gain- or loss-of-function. Electrophoresis gel mobility shift assay defined binding affinities of the mutant MTF-1's to MRE. About 25% of the loss-of-functional mutants retain wild type MRE binding affinity, which suggests that these loci are related to a trans-activation rather than DNA binding. Nuclear localization and trans-activation of MT transcription of mutant MTF-1's will be compared to wild type MTF-1. These studies further define the structure-functional relationship of MTF-1 and support the phosphorylation model for MTF-1 activation.

2092 UNDERSTANDING COBALT CHLORIDE-INDUCED LUNG INJURY THROUGH CYTOMETRIC, METABOLOMIC, PROTEOMIC, AND HISTOLOGICAL ANALYSIS OF BRONCHIOALVEOLAR LAVAGE FLUID (BALF).

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Over 1 million workers in the United States are exposed to cobalt, primarily through inhalation, leading to several disorders like respiratory tract hyperplasia, fibrosis and asthma. Since the primary target of exposure is the respiratory tract, identification of signature biomarkers of injury and inflammation specific to cobalt exposure should bolster our understanding of metal-induced toxicity at the cellular and molecular level. To study the impact of cobalt chloride-induced lung insult, seven groups of 10 weeks old mice were anesthetized and dosed, via intranasal instillation, with cobalt chloride (1 mM, 6, 24, and 48 hrs.), lipopolysaccharide (LPS, 125000 E.U., 6 hrs.), or saline (vehicle control, 6, 24, and 48 hrs.). BALF was collected and analyzed for inflammatory biomarkers, cell counts, total protein, and proteomic and metabolomic profiles. Lung lobes were also collected for RT-PCR and histological studies. Cytometric bead assay results indicate that 6 hours after dosing, both cobalt chloride and LPS show a significant increase in the inflammatory cytokines, TNF-α and IL-6, while only LPS treatment led to significant changes in cell counts. Proteomic analysis of the BALF identified several cobalt-specific (e.g. keratin complex proteins, lung surfactant convertase, 14-3-3 protein) and LPS-specific (e.g. calgranulin B, peroxiredoxin 6, and lactoferrin) markers. Metabolomic profiles revealed significant treatment specific metabolite clustering. Correlating these cytometric, metabolomic, and proteomic changes in BALF with histological and gene expression changes in the lung tissue will provide us a better understanding of toxicity of inhaled metals.

2093 CHRONIC EXPOSURE TO ZINC CHROMATE INDUCES CHROMOSOME INSTABILITY IN HUMAN LUNG CELLS.

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Hexavalent chromium (Cr(VI)) compounds are well established human lung carcinogens. Solubility plays a key role in the carcinogenicity of Cr(VI), with the most potent carcinogens being the particulate Cr(VI) compounds, however, their carcinogenic mechanism remains poorly understood. Epidemiology and animal studies indicate that zinc chromate may be the most potent chromium carcinogen. Therefore, we investigated the toxicity of zinc chromate. We found that chronic exposure to zinc chromate induced persistent chromosome damage in human lung cells with a 24 and 120 h exposure to 400 ng/cm2 inducing 49 and 43 % metaphases with damage and 81 and 70 total aberrations. Zinc chromate also induced DNA double strand breaks. For example, exposure to 400 ng/cm2 zinc chromate for 24 and 120 h induced 15 and 13 average H2A.X foci per cell above background. Chronic exposure to zinc chromate induced a concentration- and time-dependent increase in aneuploid cells progressing from no aneuploid metaphases at 24 h to 52% aneuploid metaphases after a 120 h exposure to 400 ng/cm2 zinc chromate. Chronic exposure to zinc chromate also caused metaphase damage indicative of spindle assembly checkpoint bypass with increases in centromere spreading, premature centromere division and premature anaphase. These data show that chronic exposure to zinc chromate induces both structural and numerical instability which may be attributed in part to spindle assembly checkpoint bypass. This work was supported by the Maine Center for Toxicology and Environmental Health.

2094 THE ULTRASTRUCTURAL EFFECTS OF COPPER DIMETHYLDITHIOCARBAMATE (CDDC) ON MATERNAL AND NEWBORN BRAINS OF LONG-EVANS RATS.

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The potential toxic effects on human health and deleterious effects to the environment by copper dimethyldithiocarbamate (CDDC), an alternative wood preservative to chromated copper arsenate (CCA), have not been investigated. Previous data from this lab revealed that a subacute exposure to CDDC (25, 50, and 75 mg/kg) engendered stress protein induction and lipid peroxidation in maternal hippocampi and newborn whole brains of Long-Evans rats as compared to control animals. Graphite furnace atomic absorption spectrophotometry (GFAAS) demonstrated a dose- dependent increase in copper concentrations in the tissues of treated animals as compared to controls. The bioaccumulation of copper in tissues has been linked to oxidative damage and lipid peroxidation. In this study, pregnant Long-Evans rats (220-270 g) were treated daily with 0 and 75 mg/kg CDDC by oral gavage starting from day 6 of gestation and continuing to parturition. Following parturition, maternal and newborn rats were euthanized and brain tissues were removed and processed for transmission electron microscopy. Electron microscopy revealed demyelination and by-products of peroxidative damage in treated maternal hippocampi. Treated newborn hippocampi exhibited numerous degenerating mitochondria, membrane bound inclusion bodies, and vacuoles containing electron dense material. CDDC was shown to cause neurodegeneration to maternal and newborn hippocampi. This toxicity is attributable to copper induced lipid peroxidation.

2095 THE EFFECTS OF ZIRAM ON RAT HIPPOCAMPAL ASTROCYTES.

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Ziram (zinc-bis(dimethyldithiocarbamate)) is an agricultural dithiocarbamate fungicide that is used on a variety of plant fungi and diseases and also, as an accelerator in rubber manufacturing, packaging materials, adhesives and textiles. Pesticides have been shown in several studies to be the leading candidates of environmental toxins that may contribute to the pathogenesis of several neurodegenerative diseases. The dithiocarbamate, ziram, has also been reported to cause hepatotoxicity and have adverse effects on the reproductive system as well as on the developmental process. In spite of their generally acknowledged low toxicity, dithiocarbamates are known to cause a wide range of neurobehavioral effects as well as neuropathological changes in the brain. Astrocytes play a key role in normal brain physiology and in the pathology of the nervous system. This investigation studied the effects of ziram on rat hippocampal astrocytes. Cells were maintained in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum. Astrocytes were exposed to ziram for one hour at 37 degrees C and then re-fed with complete media for 24 hours. The trypan blue exclusion assay was carried out to determine cell viability. The LC50 for ziram was determined to be 1 uM. Morphological observations, by phase contrast and scanning electron microscopy, verified the cytotoxic effects of ziram. The relationship between cytotoxicity caused by Ziram and intracellular levels of Cu and Zn was also investigated. Inductively Coupled Plasma-Optical Emission Spectroscopy showed, in all cases of treatment with Ziram, that a minimal 6 fold increase of copper levels was observed in astrocytes. Elevations of the transition metal copper may also play a role in ziram induced astrocyte toxicity.

2096 STUDY OF INFLUENCE OF COMBINED INTRODUCTION OF MANGANESE AND PIRACETAM ON COGNITIVE FUNCTIONS IN WHITE RATS.

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The study was conducted in white male rats in conditions of single and chronic (during 30 days) intragastric introduction of manganous chloride (MnCL2) dosing 50 mg/kg with drinking water. After single administration of MnCL2 the number of animals trained with the test of conditioned active avoidance reaction (CAAR) diminished by 26% vs. the control and the latent period of the reaction increased by 20%. After the chronic introduction of MnCL2 the number of trained animals as in case of the single introduction diminished by 26%, while the latent period increased by 25%. Combined single introduction of MnCL2 with Piracetam (dosing 100 mg/kg intraperitoneally) resulted in diminution of the trained animal number by 58% and the latent period increase by 35%, and in case of chronic introduction of the toxic agent and Piracetam these values worsened by 49 and 30%, respectively.Thus, intragastric introduction of MnCL2 in rats causes impairment of learning reactions, which was not alleviated with Piracetam in conditions of single and chronic administration

2097 *IN VIVO* RESPONSE TO SODIUM TUNGSTATE: A CYTOGENETIC ANALYSIS OF SPLENIC T CELLS AND PROFILE OF SYSTEMIC AND LOCALIZED CYTOKINE LEVELS.

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Tungsten (W) is widely used in military applications, such as in armor-penetrating munitions and small caliber ammunition. Recent reports suggest that under certain soil conditions, W may solubilize and has been observed at high concentrations in tap water samples in Nevada. The literature on W reveal a preferential accumulation in some immune organs, such as spleen, thymus, lung-associated lymph nodes and a potential link to clinical conditions, including some leukemia and rhabdomyosarcoma. This study evaluated chromosome damage in splenic T cells and the cytokine levels in spleen and blood following 70 consecutive days of oral dosing with 62.5 mg/kg of sodium tungstate (NaW) or dH2O (n=5/ (sex group) on adult (P0) Sprague-Dawley rats. Bioassays included effects on the balance of pro- and anti-inflammatory cytokine levels in the spleen and blood. Blood samples were drawn at 24 hours, and 14, 35 and 70 days for cytokine profiling from P0 rats in each exposure group and additional samples taken at post-natal day (PND) 70 from the offspring (F1). A Spectral Karyotype (SKY) analysis of splenic T cells for chromosomal aberrations was performed on the P0 rats at 70 days and on F1 rats at PND70. Blood concentrations of IFN-γ, IL-6, IL-4, and IL-10 were detected in P0 and F1 generations at levels similar to controls at all time points. Cytokine levels measured from supernatants of cultured spleen cell demonstrated that IL-1β, IL-4, TGF-β, TNF-α, and IFN-γ levels were either at or below non-exposed levels at all time points in both P0 and F1 rats. In addition, SKY analyses revealed several chromosomal abnormalities in the splenic T cells from the P0 rats after the 70 day exposure to NaW. Further investigation is required to determine whether a dose dependent response or longer term exposure to NaW may result in more extensive or sustained changes.

2098 PHARMACOKINETICS OF TUNGSTEN (188W) FOLLOWING ACUTE SODIUM TUNGSTATE INHALATION IN RATS.

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Aerosol cloud formation may occur when certain tungsten (W) munitions strike hard targets. The tissue dosimetry of inhaled W has been incompletely characterized. The objective of this study was to determine the pharmacokinetic of inhaled

W in rats. Male, 16-week old, CD rats underwent a single, 90-min, nose-only exposure to a Na2188WO4 aerosol (0.26 mg W/m3; MMAD 1.5 µm). Rats had the right nostril plugged to prevent nasal deposition of 188W on the occluded side. Tissues were sampled at 0, 1, 3, 7, and 21 days post-exposure and 188W concentrations determined using gamma spectrometry. Peak 188W concentrations were observed immediately following the end of the 90 min exposure and dropped appreciably in most tissues within 1 day. 188W accumulation varied among different tissues with highest concentrations seen in the lung, nasal epithelium, thyroid gland, femur, and kidney (up to 18,000 nCi/g). Urine 188W concentrations were also elevated throughout the first 7 days. Tissues with minimal 188W accumulation (peak 188W concentrations of 100 to 250 nCi/g tissue wet weight) included lung-associated lymph nodes, heart, thymus, adrenal gland, testes, pancreas, and spleen. The highest end-of-exposure brain 188W concentrations were seen in the pituitary gland (~285 nCi/g) whereas levels seen in the olfactory bulb, striatum, cerebellum, and cortex were much lower (20-50 nCi/g) suggesting that 188W is largely excluded by the blood brain barrier. 188W elimination from most tissue compartments was relatively rapid (apparent half life of elimination < 1 d). Tissues with slower terminal elimination rates included femur, lung, and spleen.

2099 TUNGSTATE'S EFFECTS ON IMMUNE AND NON-IMMUNE HUMAN CELL TYPES WHEN ANALYZED IN VITRO.

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Of great interest to both environmentalists and the United States military are the adverse health effects of using tungsten in military munitions. Unfortunately, the potential toxicological implications of using these "green bullets" are still unknown. Our initial experiments seek to determine the immunological impact of tungstate (NaW) on human immune (leukocytes) and non-immune (endothelial) cells *in vitro*. Leukocytes were exposed to NaW (0.001mM-10mM) and the mitogen ConcanavalinA (ConA) (10µg/ml) for 48 and 72 hr and analyzed for cytokine production using Cytometric bead arrays and flow cytometry. In addition, a monolayer injury assay "scratch assay" was performed using primary human endothelial cells to determine if NaW at 1mM has an effect on the migration property of cells involved in wound healing after 1, 4 and 24 hr exposure. Also, F-actin microfilament ratios were used to measure NaW's influence at 1mM-10mM concentrations on cytoskeletal reorganization. Lastly, DNA cell cycle analyses were performed on peripheral blood mononuclear cells activated by ConA (10µg/ml), using propidium iodide staining and flow cytometry to determine NaW's effect on the growth of activated immune cells. NaW effected the production of multiple cytokines, including IL-2, IL-6, MCP-1 and TNF-α. The scratch assay revealed that increasing NaW concentrations inhibit cell migration over a 48-hr period. This observation was supported by an increase in cellular F-actin ratios in a dose dependent manner. Finally, cell cycle analyses revealed that increasing NaW concentrations in the presence of activated immune mononuclear cells inhibited cell cycle progression when compared to untreated cells. These results indicate that NaW may have both direct and indirect adverse effects on cells that are attempting to carry out their immune and/or non-immune functions.

2100 ACUTE AND SUBACUTE TOXICITY OF ORALLY ADMINISTERED SODIUM TUNGSTATE IN SPRAGUE-DAWLEY RATS.

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Tungsten and tungsten "alloys" are used in military munitions. A study conducted by the Army Corps of Engineers has indicated that these materials may, under certain conditions, produce soluble forms of tungsten which are capable of entering the groundwater. The objective of these studies was to determine the relative oral toxicity of soluble sodium tungstate in male and female rats. This information will, in turn, aid in the development of an accurate reference dose for the remediation of ranges exposed to tungsten bullets and other munitions using tungsten. It was hypothesized that ingestion of soluble tungsten compounds will cause overt toxic signs in rats only at the higher dosage levels and that a No Observed Adverse Effect Level (NOAEL) can be assigned to the munition compound upon completion of these studies.

2101 HEALTH EFFECTS OF EMBEDDED FRAGMENTS OF TUNGSTEN AND TUNGSTEN ALLOYS.

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Alloys of tungsten such as Tungsten/Nickel/Cobalt (W/Ni/Co) and Tungsten/Nickel/Iron (W/Ni/Fe) are being developed as potential replacements for Depleted Uranium and as anti-personnel weapons. Two components of tungsten alloys, nickel and cobalt, are known carcinogens, and there is evidence that another component, iron, may increase the development of cancer. A preliminary study by Kalinich et al. (2005) demonstrated that rats implanted with W/Ni/Co developed aggressive, metastatic tumors, while rats implanted with Ni alone developed tumors but not metastases. These data suggest that Tungsten (W) alloys may present a unique health hazard to Soldiers wounded by W alloys. To better understand the effects of these alloys as well as tissue changes preceding or accompanying such events, we implanted Fisher-344 rats with pellets of W, W/Ni/Co or W/Ni/Fe. Effects were assessed 1, 3, 6, 12, and 24-months post-implantation. Our study confirmed the results of the Kalinich study; within 6 months Fisher 344 rats implanted with pellets of W/Ni/Co developed metastatic tumors originating at the site of implant. No rats implanted with W or W/Ni/Fe developed overt adverse effects as a result of the pellet implantation, nor were any tumors detected in these animals. These results demonstrate that the W/Ni/Co alloy is a potent carcinogen in this animal model. In order to better predict whether these and other W alloys are of concern to Soldiers, further research is needed to understand the mechanisms by which this alloy and other W alloys behave in models of fragment wounding such as the one employed in this study.

2102 REMOVAL OF TISSUE MANGANESE BY P-AMINOSALICYLIC ACID (PAS) IN MANGANESE-EXPOSED RATS *IN VIVO*.

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Para-aminosalicylic acid (PAS), an FDA-approved anti-tuberculosis drug, has been used successfully in treatment of severe Mn Parkinsonism in humans (Jiang et al., JOEM 48:644, 2006). However, the mechanism of its action remained unknown. The carboxyl-, hydroxyl-, and amino- groups in PAS structure render the molecule ideal to chelate metals. The current study was conducted to explore if PAS acted as a chelating agent to remove Mn from body fluids and tissues of rats subchronically exposed to Mn. Sprague-Dawley rats received daily i.p. dose of 6 mg Mn/kg, 5 d/wk for 4 wks, followed by daily sc. dose of PAS (100 and 200 mg/kg as the low and high dose group, respectively), 7 d/wk for another 2, 3 or 6 wks. Metal concentrations were determined by absorption spectrophotometry. Mn exposure significantly increased, to various degree, the concentrations of Mn in RBC, plasma, CSF, 6 selected brain regions and 6 organs. Following high-dose PAS treatment for 3 wks, Mn levels in liver, heart, spleen and pancreas were significantly reduced by 31.9%, 30.6%, 25%, and 33.4%, respectively (with no effect on testis and kidney). While the high-dose, 3-wk PAS treatment selectively reduced Mn levels in striatum, thalamus and choroid plexus (CP) of Mn-exposed animals, the same dose of PAS for 6 wk further reduced Mn levels in striatum, thalamus, CP, hippocampus and frontal cortex by 28.2%, 29.1%, 27.4%, 23.2%, and 15.5% ($p<0.05$), respectively. PAS treatment also significantly reduced Mn concentrations in the RBC (22.1%) and CSF (28.3%) with no effect on plasma Mn levels. Mn exposure increased Fe concentrations in RBC, CSF, selected brain areas and liver. Therapy with high-dose PAS for 3 wks reduced Fe levels in the CSF, striatum, choroid plexus, hippocampus, and liver by 47.1%, 16.4%, 20%, 46.9%, and 33.8%, respectively, to the levels that were close to control values. These data suggest that PAS likely acts as a chelating agent to mobilize metals in tissue and therefore remove them from the body. (NIEHS ES08146 and DoD W81XWH-05-1-0239)

2103 THE EFFECT OF MANGANESE ACCUMULATION ON GLUTATHIONE METABOLISM IN RAT LIVER SUBCELLULAR FRACTIONS.

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Glutathione (GSH) is an endogenous antioxidant involved in cellular defense against oxidative stress. Manganese (Mn) toxicity is thought to be initiated by oxidative stress and associated with a decrease in GSH. The purpose of this study was to examine the effect of Mn on enzymes involved in GSH metabolism in cytosolic and microsomal fractions of rat liver. Male Sprague-Dawley rats were fed normal (n = 22) or iron deficient (n = 22) diets and half had access to water supplemented with or without Mn (1gMn/L d.d. water). After six weeks, rats were sacrificed and livers were collected; cytosolic and microsomal fractions were obtained via ultracentrifugation. Glutathione S-transferase (GST), GSH peroxidase (GPx), and GSH reductase (GR) activity were assessed and Mn concentrations were measured in each

fraction. Rats supplemented with Mn (CNMn and IDMn) showed a significant increase in both cytosolic and microsomal Mn concentration compared to CN ($p < 0.001$). The cytosol accumulated 28% more Mn then the microsomal fraction. Cytosolic GST activity significantly decreased in IDMn compared to CN (83 and 103 U/mg protein respectively, $p < 0.05$) and microsomal GST activity was significantly higher in CNMn compared to CN (54 and 39 U/mg protein respectively, $p < 0.01$). In the cytosol, there was a negative correlation between both GST and GPx activity and Mn accumulation ($r = -0.32$ and -0.29, respectively $p < 0.05$) and a positive correlation in GR activity ($r = 0.32$, $p < 0.05$). No such observation was observed in the microsomal fraction. Manganese accumulation appeared to alter GSH metabolism in the microsomal and cytosolic subcellular fractions of the liver. At moderate Mn accumulation (CNMn) microsomal GST increased but as Mn accumulation became more severe (IDMn), GST activity was suppressed. The correlational relationships observed suggest that Mn accumulation in the cytosol inhibited both GST and GPx activity and stimulated GR activity. These data suggest that increased GR activity due to excess Mn may serve as a protective mechanism by generating GSH.

2104 CHARACTERIZATION OF MANGANESE-INDUCED PRODUCTION OF REACTIVE OXYGEN SPECIES IN MICROGLIA: RELEVANCE TO DOPAMINERGIC NEURODEGENERATION.

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Elevated environmental and occupational exposure to manganese is known to cause the manifestation of a movement disorder called manganism. Furthermore, elevated environmental exposure to manganese may contribute to the development of idiopathic Parkinson's disease (PD) as part of the multi-factorial etiology of the degenerative disease. Analysis of postmortem PD brains and toxicant-induced animal PD models supports an important role for glial cells, especially the resident brain immune cells, microglia, in the progressive neurodegenerative process. Cultured rat microglia exposed to manganese chloride resulted in a time (1-24 hr) and concentration (1-33 μM)-dependent release of hydrogen peroxide. Comparison of the potency of various divalent metals (10 μM) in inducing hydrogen peroxide release in immortalized rat HAPI microglial cells indicated that divalent manganese (manganese chloride) was most potent (260% of control). In contrast, divalent cadmium (170%), mercury (140%) and copper (130%) were less effective than divalent manganese and divalent iron and zinc were totally ineffective in inducing microglial hydrogen peroxide release. In primary rat neural cell cultures, manganese chloride was more effective in causing the degeneration of dopaminergic neurons in microglia-rich mixed neuron-glia cultures than in microglia-poor neuron-enriched cultures. The microglia-enhanced dopaminergic neurotoxicity of manganese chloride was significantly reduced by glutathione or catalase. These results suggest that the manganese chloride-stimulated microglial ROS production contributes to dopaminergic neurotoxicity of manganese.

2105 ALTERED COPPER TRANSPORT AT RAT BLOOD-CSF BARRIER FOLLOWING SUBCHRONIC EXPOSURE TO MANGANESE.

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Disordered copper (Cu) homeostasis has been implicated in the pathogenesis of idiopathic Parkinson's disease. Our recent studies have shown that subchronic exposure to Mn in rats results in an increase in Cu levels in brain tissue, cerebrospinal fluid (CSF) and choroid plexus (CP); the latter comprises the blood-CSF barrier (BCB). Since the BCB plays a key role in maintaining Cu homeostasis in the CSF, we hypothesized that accumulation of Mn in the CP altered Cu transport, leading to an elevated Cu in the central milieu. Rats received daily i.p. dose of 6 mg Mn/kg (as MnCl2), 5 d/wk for 4 wks. At the end of treatment, rats were subjected to in situ ventriculo-cisternal perfusion operation. [64Cu] (0.4 uM as CuCl2, 3.5 uCi/mL) and a space marker [14C]sucrose were concomitantly infused into the brain ventricle and the CSF was collected via a cisternal cannula. At the steady state, the radioactivities in the outflow CSF as the % of infused dose were 15.6+SE0.80% and 115+17% for [64Cu] and [14C]sucrose, respectively, in control rats. Mn exposure did not affect the steady state [14C]sucrose (122+3.6%), but it increased [64Cu] in the outflow CSF by 2.6 fold (40.3+1.5%, n=3) as compared to controls ($p<0.001$), suggesting a significant reduction in Cu uptake and/or removal by the CP. We further used choroidal epithelial Z310 cells to determine the effect of Mn on Cu transporter proteins, i.e., ATP7A and ATP7B, which functions to oust intracellular Cu. Z310 cells were incubated with 200 μM Mn for 6 h. The expression of ATP7A and ATP7B was quantified by real-time RT-PCR and Western blot. Mn exposure significantly decreased ATP7A and ATP7B RNAs by 50% and 57%, respectively, as compared to controls. Similar decreases were seen in protein expression. Taken together, it appears likely that Mn exposure slows down the Cu clearance from the CSF by altering Cu transporter function in the BCB. Questions as to why Mn exposure increases Cu in the CP, what exact mechanisms underlie altered Cu homeostasis in the CSF, and how this may contribute to Mn parkinsonism, remain to be explored. (NIH/NIEHS ES08146)

2106 COPPER IS REQUIRED FOR HYPOXIA-INDUCIBLE FACTOR-1 ACTIVATION.

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Previous studies have demonstrated that copper (Cu) up-regulates hypoxia-inducible factor 1 (HIF-1). The present study was undertaken to test the hypothesis that Cu is required for HIF-1 activation. Treatment of HepG2 cells with a Cu chelator tetrathylenepentamine (TEPA) or siRNA targeting Cu chaperone for superoxide dismutase 1 (CCS) suppressed hypoxia-induced activation of HIF-1. Addition of excess Cu to the cultures relieved the suppression by TEPA, but not by CCS gene silencing, indicating the requirement of Cu for the activation of HIF-1, which is CCS-dependent. Cu deprivation did not affect the production or stability of HIF-1α, but reduced HIF-1α binding to the hypoxia-responsive element (HRE) of the target genes and to p300, a component of HIF-1 transcriptional complex. Cu likely inhibits the factor inhibiting HIF-1 (FIH-1) to ensure the formation of HIF-1 transcriptional complex. This study thus defines that Cu is required for HIF-1 activation through regulation of the binding of HIF-1α to the HRE and the formation of the HIF-1 transcriptional complex.

2107 COPPER SUPPLEMENTATION REVERSES CARDIOMYOCYTE HYPERTROPHY THROUGH A DIRECT REDUCTION IN THE CELL SIZE.

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Previous studies showed that copper supplementation reversed heart hypertrophy induced by pressure overload in a mouse model. The present study was undertaken to understand the mechanism for copper-induced regression of cardiac hypertrophy. Primary cultures of neonatal rat cardiomyocytes were treated with phenylephrine (PE, 100 μM) in cultures to induce cellular hypertrophy. The hypertrophied cardiomyocytes were exposed to copper sulfate (5 μM) in cultures. The purity of cardiomyocytes in the cultures was more than 95%, as determined by fluorescence-activated cell sorting of α-sarcomeric actin stained cells, excluding the effects of fibroblasts and other cells. The size of the hypertrophied cardiomyocytes was reduced by copper treatment, as measured by flow cytometer. However, copper did not change the phenotype of non-PE-treated control cells. Morphological studies indicated copper reversed the PE-induced sarcomere reorganization in cardiomyocytes. DNA content and p-Histone 3 were not changed after copper treatment, indicating no cell division occurred in the regression of cell hypertrophy. The cell number in the PE-treated cultures was less than that in the non-PE-treated controls. Copper increased the cell number in the PE-treated cultures, suggesting that copper might have protective effect on cell death. Because vascular epithelial growth factor (VEGF) is involved in copper-induced regression of heart hypertrophy in vivo, VEGF in the copper-induced regression of myocyte hypertrophy in cultures was examined. Anti-VEGF antibody was added to the cultures (2 ng/ml) one hour before copper addition. In contrast, VEGF was used to replace copper (200 ng/ml). In the presence of anti-VEGF antibody, copper-induced regression of cell hypertrophy was blunted, and VEGF alone reversed cell hypertrophy as the same as copper did. This study thus demonstrates copper directly reduces the size of hypertrophied cardiomyocytes leading to regression of cardiac hypertrophy, a process that is VEGF-dependent. Supported in part by NIH grants HL63760 and HL59225.

2108 APOPTOSIS GENE EXPRESSION IN HUMAN EPIDERMAL KERATINOCYTES TREATED WITH SODIUM ARSENITE USING REAL TIME PCR ARRAY.

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Arsenic exposure via contaminated drinking water is a great public health concern worldwide. Chronic arsenic exposure has been associated with human skin, lung and bladder cancer and other chronic effects. We have previous reported that sodium arsenite stimulated cell proliferation at low dose (peaked at 0.5 μM), and

induced apoptosis at high doses (starting at 1.0 μM and up to 40 μM) in human epidermal keratinocytes (HaCaT). The mechanisms of apoptosis induced by arsenic are not completely understood. This study was to investigate arsenic effects on expression of the genes relevant to apoptosis and examine the possible mode of action by arsenic in human cells. HaCaT cells were treated with 0 μM (controls), 0.5 μM, 1.0 μM and 10 μM of sodium arsenite for 72 hours. Total RNA was isolated and gene expression of 93 genes relevant to apoptosis and 3 endogenous controls was assayed using real time PCR array (Apoptosis Panel of Taqman Low Density Array, ABI). Target gene expression was normalized to 18s RNA and calculated as fold of the controls. The results showed most of apoptosis-related gene expression tested increased at 0.5 μM and 1.0 μM and decreased at 10 μM. The anti-apoptosis genes such as BIRC2 and BIRC3 greatly increased (3.3 and 3.2 folds of control, respectively) at the lowest dose, 0.5 μM, but decreased at 1 μM. Whereas, the pro-apoptosis genes such as BAX, PMAIP1 and BNIP3L gradually increased at 0.5 μM and peaked at 1 μM (3.4, 2.0, and 2.4 folds of control, respectively). Other apoptosis-related genes, TNF-α and the NFκB pathway genes (IKKα, NFκB2, IκBε), also showed dose- response relationships and peaked at 1 μM (5.3, 4.6, 2.2 and 2.0 folds of control, respectively). These results showed that depending on concentrations, arsenic can function both as anti-apoptotic and apoptotic agent in human cells. (This abstract does not reflect EPA policy.)

2109 EFFECTS OF GLUTATHIONE AND HYDROGEN PEROXIDE ON THE STABILITY OF BILIARY METABOLITES OF INTRAVENOUSLY INJECTED ARSENITE IN RATS.

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Trivalent arsenicals are more toxic than analogous pentavalent arsenicals; arsenite (iAs^{III}) vs arsenate (iAs^{V}), monomethylarsonous acid (MMA^{III}) vs monomethylarsonic acid (MMA^{V}), and dimethylarsinous acid (DMA^{III}) vs dimethylarsinic acid (DMA^{V}). It has been reported that arsenic-glutathione (As-GSH) complexes such as arsenic triglutathione (ATG) and methylarsenic diglutathione (MADG) were major metabolites in the rat bile after intravenous administration of iAs^{III}. Recently, we showed that both ATG and MADG were unstable and were easily hydrolyzed to iAs^{III} and MMA^{III}, respectively, and MMA^{III} was oxidized to MMA^{V} in bile. We postulated that the oxidation of trivalent arsenicals plays a critical role in the detoxification of arsenicals in bile. In the present study we report effects of GSH and H_2O_2 on the stability of As-GSH complexes in bile. Male SD rats were injected intravenously with saline or iAs^{III} at a dose of 2.0 mg As/kg body weight, and bile fluid was collected on ice for 30 min. To estimate stability of As-GSH complexes in bile, ATG or MADG was added in bile and then incubated at 37 °C for 10 min with or without catalase. Concentrations of biliary H_2O_2 and GSH in the arsenic-treated rats were 12.6 and 4.6 times higher than the control value, respectively. Exogenously added MMA^{III} were oxidized to MMA^{V} in bile depending on the concentration of H_2O_2 in bile. Catalase prevented oxidation of trivalent arsenicals to the corresponding pentavalent arsenicals. Exogenously added GSH stabilized As-GSH complexes in bile. These results suggest that GSH prevents hydrolysis of As-GSH complexes and the generation of unconjugated toxic trivalent arsenicals, whereas H_2O_2 converts trivalent arsenicals to less toxic pentavalent arsenicals.

2110 ARSENITE MODIFIES P53-MEDIATED RESPONSE TO CISPLATIN-INDUCED DNA DAMAGE IN OVARIAN CANCER CELLS.

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DNA repair is an important mechanism of resistance of ovarian cancer cells to the cancer drug cisplatin. Cisplatin causes bulky DNA-adducts that are primarily repaired by the nucleotide excision repair (NER) pathway. Global genomic repair (GGR) is the subpathway of NER that removes lesions from the entire genome. XPC and DDB2 are key DNA damage recognition proteins in GGR. Stabilization of p53 after DNA damage transcriptionally activates $p21^{CIP1/WAF1}$, XPC and DDB2. $p21^{CIP1/WAF1}$ is a cyclin-dependent kinase inhibitor which causes cell cycle arrest that allows time for damaged DNA repair. Phosphorylation of Rb in G1 is essential for G1 to S progression. Previous data in our lab indicated that arsenic potentiates cisplatin cytotoxicity with concurrent treatment with hyperthermia. We therefore hypothesized that arsenite modifies the response of p53 regulated genes including $p21^{CIP1/WAF1}$, XPC and DDB2 after cisplatin induced DNA damage in ovarian cancer cells. Cisplatin-sensitive A2780 and -resistant CP70 cells were treated with cisplatin with or without arsenite at 37 °C or 39 °C for 1 h. Stabilization of p53 phosphorylated on Ser15 correlated with the induction of XPC and DDB2. Both cell lines showed similar levels of induction. No significant effect was noticed with arsenite treatment. However, arsenic caused a delay in $p21^{CIP1/WAF1}$ induction in cisplatin treated CP70 cells when compared to stronger and earlier induction in A2780 cells. Levels of pRb phosphorylation on Ser807/811 were relatively low in CP70 cells suggesting G1 cell cycle arrest. Data for the concentration-dependent stabilization of p53 phosphorylated on Ser15, $p21^{CIP1/WAF1}$, pRb phosphorylated on Ser807/811, DDB2 and XPC suggested that CP70 cells require higher concentrations of cisplatin to stabilize these proteins. Our future work will be to measure cellular accumulation of cisplatin and determine the rate of removal of cisplatin-DNA adducts using ICP-MS. Supported in part by NIH grants 1P30ES014443 and 5R01ES011314.

2111 EXPOSURE TO ARSENIC DISRUPTS VEGF-DIRECTED VASCULOGENESIS CAUSING SUBSEQUENT PLACENTAL INSUFFICIENCY AND SPONTANEOUS ABORTION.

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Human populations subject to arsenic exposure through drinking water experience an increased the frequency of miscarriage. Experimental mouse studies in our lab are consistent with the human condition showing that arsenic (AsIII) exposure causes spontaneous abortion between E7.5 and E12.5. Placental morphogenesis involves stromal cell secretion of vasculotrophic factors in the VEGF family that bind cell surface (VEGF) receptors (R1 & R2) to direct angioblast differentiation, proliferation, adhesion and apoptosis during placental vasculogenesis. AsIII exposure data in cultured endothelial cells in our lab show endothelial dysfunction in the form of increased Flt-1(R1) expression and decreased Flk-1(R2) expression. In addition, we have demonstrated AsIII mediated disruption of endothelial cell adhesion through the protein kinase C-α (PKCα) pathway. Overall, these results suggest that AsIII exposure alters the VEGF regulatory pathway during placental vasculogenesis. Disruption of placental vasculogenesis then results in placental insufficiency and spontaneous abortion. The goal of the project is to conduct a functional analysis on the VEGF receptors thereby revealing the molecular mechanisms for how AsIII exposure disrupts placental vasculogenesis and function. The results from this project suggest that arsenic toxicity may be a critical environmental risk factor for pregnancy complications of unknown etiology. Much of what we know about developmental biology has been learned through the study of abnormalities. In that context, our studies in arsenic toxicity provide a unique means for selective perturbation of the VEGF regulatory system resulting in new perspectives on neovascularization in development and disease.

2112 SENSITIVITY TO SODIUM ARSENITE DEPENDS UPON A FUNCTIONAL SPINDLE CHECKPOINT.

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Sensitivity to arsenic, a chemotherapeutic for acute promyelocytic leukemia, was evaluated in human malignant melanoma cell lines. Cell lines sensitive to pharmacological concentrations of arsenite arrested in mitosis. Mitotic arrest was associated with spindle checkpoint activation suggested by BubR1 phosphorylation and stabilization of securin and cyclin B. Arsenite also increased activating CDK1 Thr161 phosphorylation while decreasing inhibitory Tyr15 phosphorylation. Mitotic arrest resulted in apoptosis as indicated by colocalization of mitotic phospho-Histone H3 with active caspase 3. Apoptosis was associated with BCL-2 Ser70 phosphorylation. Inhibition of CDK1 with roscovitine in arsenite-treated mitotic cells inhibited spindle checkpoint maintenance as inferred from reduced BubR1 phosphorylation, cyclin B expression, and diminution of mitotic index. Roscovitine also reduced BCL-2 Ser70 phosphorylation and protected against apoptosis, suggesting mitotic arrest may cause hyperactivation of CDK1 which directly or indirectly leads to BCL-2 phosphorylation and apoptosis. A lack of mitotic arrest was observed in cell lines resistant to sodium arsenite. ICP-MS analysis indicated resistance was not due to reduced intracellular accumulation of arsenic but rather intrinsic cellular properties. Consequently, we evaluated spindle checkpoint function by treating arsenite-resistant cell lines with the antimitotic drug paclitaxel and found the checkpoint to be dysfunctional. The weakened spindle checkpoint was due to reduced expression of BubR1. We tested the ability of siRNA BubR1 knockdown to confer arsenite resistance in an arsenite-sensitive cell line. BubR1 suppression inhibited arsenite-induced mitotic arrest and prevented apoptosis at low arsenite concentrations. The current study indicates the spindle checkpoint is an important target of arsenic and spindle checkpoint competence may be a prerequisite for a positive response to arsenic's clinical application. Supported in part by ES011314, ES014443 & ES011564.

2113 EXPRESSION OF KERATIN 6A AND KERATIN 16 IN ARSENITE AND CADMIUM TRANSFORMED UROTSA CELL LINES IS INDUCED *IN VIVO* AND IS ASSOCIATED WITH SQUAMOUS DIFFERENTIATION.

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This laboratory has previously demonstrated that cadmium (Cd^{+2}) and arsenite (As^{+3}) transformed UROtsa cells grown on serum-containing growth medium and the derived heterotransplants all overexpressed keratin 6a mRNA and protein compared to parental UROtsa cells grown in serum-containing growth medium. Growth factor deletion and addition studies indicated that the level of keratin 6a expression was regulated by the presence of both insulin and epidermal growth factor. In contrast, growth factors had no effect on the elevated levels of keratin 6a expression found in transformed UROtsa cells. Subsequently, this laboratory generated independently, additional As^{+3} and Cd^{+2} transformed cell lines. In the present study, the expression of keratin 6a and 16 was determined in these transformed UROtsa cell lines grown in serum containing media and the tumor heterotransplants. Real time PCR and western analysis was used to determine gene and protein expression in the transformed cell lines and heterotransplants. Immunohistochemistry was performed on the tumor heterotransplants. The data obtained indicates that parental UROtsa cells express low levels of keratin 6a and 16. However, the process of transformation induces the expression of keratin 6a and 16 in some of the As^{+3} and Cd^{+2} transformed cell lines. The tumor heterotransplants generated from these cell lines all expressed keratin 6a and 16. Immunohistochemical analysis of these tumors indicated that the expression of keratin 6a and 16 was localized to the area of the tumor with squamous differentiation. In conclusion, our data suggests that there is a gain in expression of keratin 6a and 16 when the transformed cell lines are grown as heterotransplants, and the expression is associated with squamous differentiation.

2114 CHARACTERIZATION OF TUMOR HETEROTRANSPLANTS PRODUCED FROM INDEPENDENTLY GENERATED CLONES OF ARSENITE AND CADMIUM TRANSFORMED HUMAN UROTHELIAL CELLS.

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Arsenite (As^{+3}) and cadmium (Cd^{+2}) are known human carcinogens that can cause bladder cancer. Previous work from this laboratory has shown that both cadmium and arsenite can directly malignantly transform the UROtsa cell line and that the tumor heterotransplants produced from these transformed cells have histologic features consistent with human bladder cancer. This work was further extended in this study, and independently seven arsenite and eight cadmium transformed cell lines were generated from the parent UROtsa cells. The growth rates and morphological features were noted for each of these lines. Furthermore, these lines were injected into nude mice and the growth rates and histological characteristics were observed in the resulting tumors. The arsenite transformed cell lines had doubling times in the range of 14.18 to 33.3 hrs whereas the cadmium transformed lines had doubling times between 16.1 to 27.1 hrs. Histologically, within each metal group the tumors were similar to each other with varying levels of squamous differentiation ranging from mild/moderate to prominent. In 5 of the 7 As^{+3} generated tumor heterotransplants, there was mild squamous differentiation, whereas the other 2 heterotransplants had prominent squamous differentiation. In the Cd^{+2} group, 4 of the 8 heterotransplants had prominent squamous differentiation, whereas the rest had mild/moderate differentiation. In conclusion, the independently generated tumor heterotransplants from both arsenite and cadmium transformed UROtsa cell lines have histological characteristics and growth rates that are very similar to each other.

2115 METHYLATED BISMUTH, BUT NOT BISMUTH CITRATE OR BISMUTH GLUTATHIONE INDUCES CYTO- AND GENOTOXIC EFFECTS IN HUMAN CELLS *IN VITRO.*

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Bismuth compounds are widely used in industrial processes and products. In medicine, bismuth salts have been applied in combination with antibiotics for the treatment of Helicobacter pylori infections, for the prevention of diarrhoea and in radioimmunotherapy. In the environment, bismuth ions can be biotransformed to the volatile bismuth compound trimethylbismuth by methanobacteria. Preliminary studies have indicated that bismuth ions are methylated in the human colon by intestinal microflora following ingestion of bismuth containing salts. Information concerning cyto- and genotoxicity of these biomethylated products is limited. In the present study we investigated the cellular uptake of an organic bismuth compound (monomethylbismuth(III), (MeBi(III)) and two other bismuth compounds (bismuth citrate (Bi-Cit) and bismuth glutathione (Bi-GS)) in human hepatocytes, lymphocytes and erythrocytes using ICP-MS. We also analyzed the cyto- and genotoxic effects of these compounds. Our results show that the methylbismuth compound was better taken up by the cells than Bi-Cit and Bi-GS. MeBi(III) was best taken up by erythrocytes, followed by lymphocytes and hepatocytes. Cytotoxic effects of MeBi(III) were detectable in erythrocytes at concentrations of >4 μM, in hepatocytes at >130 μM and in lymphocytes at >430 μM after 24h exposure. Cytotoxic effects of Bi-Cit and Bi-GS were low or not detectable up to a concentration of 500 μM. Exposure of lymphocytes to MeBi(III) resulted in increased frequencies of chromosomal aberrations (CA) and sister chromatid exchanges (SCE), whereas Bi-Cit and Bi-GS induced neither CA nor SCE. These data suggest that biomethylation of bismuth ions by the intestinal microflora of the human colon leads to an increase in the toxicity of the primary bismuth salt.

2116 CO-ADMINISTRATION OF COBALT CHLORIDE PROTECTS AGAINST LIVER INJURY INDUCED BY CADMIUM CHLORIDE IN MICE.

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Administration of cadmium chloride ($CdCl_2$) to animals causes acute toxicity such as liver injury and testicular hemorrhage. Metallothionein (MT) is a well-known endogenous factor that can protect against liver toxicity of cadmium. However, little is known concerning the protective role of cobalt compounds against cadmium toxicity. To clarify the role of cobalt compounds against cadmium toxicity, we injected cobalt chloride ($CoCl_2$) simultaneously with $CdCl_2$ to male ICR mice, and examined liver injury and expression of the related genes. Co-administration of $CoCl_2$ dose-dependently reduced plasma AST activity. Histopathological examination also confirmed the protection by $CoCl_2$ against cadmium-induced liver injury. The protective effects of $CoCl_2$ were also observed in MT-I, II knockout mice, suggesting that the effect of $CoCl_2$ is not mediated by MT induction. In addition, protective effect of $CoCl_2$ was not diminished by heme oxygenase 1 (HO-1) inhibitor, tinprotoporphyrin, suggesting that the effect of $CoCl_2$ was not mediated by HO-1 induction. Since several inflammatory cytokines are known to be involved in liver injury, we examined expression of interleukin-6 (IL-6). Administration of $CdCl_2$ increased mRNA levels of IL-6 in the liver and plasma levels of IL-6 protein. However, co-administration of $CoCl_2$ did not reduce the expression of IL-6. On the other hand, administration of $CoCl_2$ alone resulted in high expression of IL-6. Interestingly, however, cadmium-induced production of serum amyloid A, which is an acute-phase protein induced by IL-6, was dramatically reduced by co-administration of $CoCl_2$. The expression of serum amyloid A is mediated by activation of STAT3, NF-κB and MAP kinases. The results of our study suggest that the inhibition of these signal transduction pathway may be involved in the cobalt-induced protection against cadmium hepatotoxicity.

2117 CELLULAR VITAMIN C INCREASES CHROMATE TOXICITY *VIA* A DEATH PROGRAM REQUIRING MISMATCH REPAIR BUT NOT P53.

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The reduction of carcinogenic Cr(VI) by low molecular weight thiols is required for activation and induction of DNA damage. Previous studies have indicated that in vivo ascorbate (Asc) is the main reducer of Cr(VI). This reductive metabolism results in the formation of ternary Asc-Cr(III)-DNA adducts and binary-Cr(III)-DNA adducts. Human cells in culture are severely Asc deficient, which results in distorted metabolism and potentially abnormal responses to Cr(VI). We found that restoration of physiological Asc levels in human lung cells increased clonogenic lethality and apoptosis by Cr(VI) as seen by enhanced activity of caspases 3 and 7 and cleavage of PARP. Enhanced cytotoxicity in mass cultures was more evident after normalization for lower Cr uptake caused by leakage of Asc into media. Delivery of Asc after Cr(VI) exposure did not change uptake-adjusted yields of Cr-DNA adducts or cytotoxicity. Protein and Ser-15 phosphorylation levels of p53 did not show any association with the presence of Asc and there were no increases in p53-driven reporter activity in Cr-treated cells. Stable silencing of p53 expression by short hairpin RNA (shRNA) had no effect on toxicity of Cr(VI) in both –Asc and +Asc IMR90 and H460 cells. However, depletion of essential components of MutS or MutL mismatch repair complexes with shRNA greatly improved survival

of all Cr-treated cells and eliminated Asc-potentiated effects on cell death. Our findings indicate that Asc plays a dual role in Cr(VI) toxicity: protective outside and potentiating inside the cell.

2118 V-PROLI/NO, A NITRIC OXIDE DONOR PRODRUG, PROTECTS LIVER CELLS FROM ARSENIC-INDUCED TOXICITY AND APOPTOSIS.

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Inorganic arsenic shows great promise in human cancer chemotherapy, although arsenic-induced hepatotoxicity is a major limiting side effect. 1-[2-(Carboxylato)pyrrolidin-1-yl]diazen-1-ium-1,2-diolate (V-PROLI/NO) is a nitric oxide (NO) donor prodrug that is metabolized by liver cytochromes P450 to release NO. Other NO-releasing agents have been shown to mitigate arsenic toxicity. Thus, the effects of V-PROLI/NO pretreatment on the toxicity of arsenic (as NaAsO2) were studied *in vitro* in a human liver (HepG2) cell line. HepG2 cells acted upon the prodrug to release NO, as assessed by nitrite levels, in a dose- and time-dependent manner to a maximal levels of 57-fold above basal levels. V-PROLI/NO also increased CYP2E1 expression in a dose-dependent manner that was directly related to the level of NO produced. In cells pretreated with V-PROLI/NO (200 μM, 24 h) then exposed to arsenic for an additional 24 h, arsenic was much less toxic (LC_{50} = 151.9 ± 5.9 μM) than in control cells (LC_{50} = 90.5 ± 6.5 μM) and the reduced cytolethality was directly related to the level of NO produced. Additionally, V-PROLI/NO pretreatment markedly reduced arsenic-induced apoptosis as measured by DNA fragmentation. Activation of mitogen-activated protein kinases (MAPKs) is a well recognized effect of arsenic and pretreatment with V-PROLI/NO suppressed phosphorylation of both ERK1/2 and JNK1/2 after arsenic exposure. V-PROLI/NO alone modestly increased metallothionein (MT), a metal-binding protein important in arsenic tolerance, but V-PROLI/NO pretreatment greatly enhanced arsenic induction of MT. Thus, the prodrug, V-PROLI/NO, protects against arsenic toxicity in human liver cells in culture, reducing cytolethality, apoptosis and dysregulation of MAPKs, through generation of NO formed after metabolism by probably CYP2E1. The potential of V-PROLI/NO for prevention of hepatotoxicity associated with arsenical chemotherapy in vivo deserves study.

2119 ELIMINATION OF THIOL CONJUGATES OF INORGANIC MERCURY AND THE ROLE OF THE MULTIDRUG RESISTANCE PROTEIN 2.

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Inorganic mercury (Hg^{2+}) forms high affinity conjugates with thiol-containing molecules such as cysteine (Cys) and homocysteine (Hcy). These conjugates are taken up by proximal tubular cells, following which Hg^{2+} becomes available to bind to chelators, such as 2, 3-dimercaptopropane-1-sulfonic acid (DMPS) or meso-2, 3-dimercaptosuccinic acid (DMSA). Previous data indicate that cellular elimination of mercuric-DMPS or -DMSA complexes likely occurs through via the multidrug resistance protein, Mrp2. In the current study, we test the hypothesis that Mrp2, localized in the luminal plasma membrane of proximal tubular cells, mediates the export of DMPS- or DMSA-*S*-conjugates of Hg^{2+} into the tubular lumen when the Hg^{2+} is taken up as a thiol-conjugate. To test this hypothesis, Wistar and TR^- (Mrp2-deficient) rats were injected with 0.5 μmol/kg $HgCl_2$ (containing ^{203}Hg) conjugated to 1.25 μmol/kg Cys or Hcy. Rats were then treated with saline, DMPS, or DMSA. In saline-treated rats, the renal and hepatic burden of Hg^{2+} was greater in TR^- rats than in controls. Accordingly, the content of Hg^{2+} in the urine and feces was less in the TR^- rats than in controls. Following treatment with DMPS or DMSA, the renal and hepatic content of Hg^{2+} in both groups of rats was significantly reduced accompanied by a significant increase in the urinary and fecal excretion. Interestingly, the change in Hg^{2+} content in the samples from TR^- rats was not as great as that of the controls. These data indicate that Mrp2 is involved in the DMPS- and DMSA-mediated elimination of Hg^{2+} from proximal tubular cells.

2120 REACTIVE OXYGEN SPECIES SCAVENGERS REDUCE HYPERPROLIFERATION AND ANCHORAGE INDEPENDENT GROWTH OF UROTSA CELLS CHRONICALLY EXPOSED TO MONOMETHYLARSONOUS ACID.

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UROtsa cells exposed to 50 nM MMA^{III} for 52 wk (MSC52) achieved hyperproliferation, anchorage independent growth, and enhanced tumorgenicity. MMA^{III} has been shown to induce reactive oxygen species (ROS), which can lead to oxidative stress response and DNA damage. It is hypothesized that after chronic MMA^{III} treatment, the cellular systems in place to protect against ROS (e.g. superoxide dismutase- CuZn-SOD and Mn-SOD) are overwhelmed by the long-term exposure to ROS, leading to chronic oxidative stress, which can lead to signaling pathway perturbations. Previous research established the acute activation of MAPK signaling cascade by ROS produced by MMA^{III} as well as chronic up regulation of COX-2 and EGFR in MSC52 cells. To establish if ROS played a role in the chronic pathway perturbations, activation of Ras, shown to both cause an increase in superoxide ($O_2^{\cdot-}$) and also be activated by increases in $O_2^{\cdot-}$, was determined in UROtsa cells exposed to MMA^{III} for 0-12 mo. Ras activation increased through 12 mo, but most dramatically after 5 mo exposure, making ROS important to study. Several studies have demonstrated that cancer cells have lowered MnSOD protein and activity. Increasing levels of MnSOD, a possible tumor suppressor, has been shown to suppress the malignant phenotype of cells. Mn-SOD protein drastically decreased after 6 mo continual exposure to 50 nM MMA^{III}. To determine if the decreased SOD played a role in transformation, SOD was added to MSC52 cells resulting in slower proliferation rates (doubling time= 42 h vs 31 h). ROS scavengers of $^1O_2$, $^{\cdot}OH$, downstream products of $O_2^{\cdot-}$ also slowed proliferation rates of MSC52 cells. To further substantiate the importance of ROS in the transformation of MSC52 cells, anchorage independent growth was assessed after the addition of ROS scavengers. Scavengers of $^1O_2$, $^{\cdot}OH$, and $O_2^{\cdot-}$ all blocked the colony formation of MSC52 cells. These data support a role for ROS in transformation of UROtsa cells exposed to MMA^{III}.(NIH ES04040, ES06694, ES07091)

2121 ALPHA-TOCOPHEROL AVOIDS OVEREXPRESSION OF OCLUDIN AND DISORDER PATTERN OF CLAUDIN-2 LOCATION, AND MANTAINS PROXIMAL RENAL FUNCTION IN MICE EXPOSED TO DICHROMATE.

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Tight junctions (TJ) regulate the ions and water selective passage across the paracellular pathway in epithelial and endothelial tissue. Ocludin, and claudin-2, are tetraspan membrane protein of TJ. Previous studies have been show that oxidative stress compromises structure and function of TJ. Cr^{6+} is a well-established oxidative stressor. Our aim was to evaluate the role of oxidative damage on ocludin and claudin-2 expression and distribution pattern and renal function by Cr^{6+} exposure. We also studied the effect of co-administration of α-tocopherol, an antioxidant, during acute administration of Cr^{6+}. Wistar female rats received potassium dichromate (15 mg/kg, SC, single dose) with or without α-tocopherol (125 mg/kg/day, by gavage) and were evaluated on days 0, 2, y 7. Renal function along the nephron was evaluated by different effect markers. Ocludin and claudin-2 were evaluated by immunohistochemical and western-blot analysis on proximal tubular cells isolated by Percoll gradient. As we expected, immunofluorescence of both proteins showed a clear presence at the apical cell borders of proximal tubules in slices of control group. This pattern was severely disorganized on day 2 after Cr^{6+} exposure and was partially recovered on day 7 for both proteins. In addition, to disorder on location pattern, ocludin was overexpressed in kidney slices of rats administered with Cr^{6+} this result was confirmed by western-blot analysis. In agreement with morphological alterations, oxidative damage and all parameters of renal function were maximally altered on day 2 and thereafter recovery ensued. Moreover, our results showed that, α-tocopherol treatment avoided oxidative damage on renal cortex and both morphological and functional deleterious effects on proximal tubule. These results suggest that α-tocopherol treatment may be use for prevent the morphological and functional deleterious effects induced by Cr^{6+} exposure.

2122 THE ROLE OF NADPH OXIDASE *VERSUS* MITCHONDRIA IN ARSENIC TRIOXIDE-INDUCED APOPTOSIS.

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Arsenic trioxide (ATO) induces reactive oxygen species (ROS), but it is unclear by what mechanism and the role ROS play in ATO-induced apoptosis. We have previously shown that sensitivity to ATO can be enhanced by glutathione depletion and can be rescued by N-acetyl cysteine. In addition, we have shown that several "antioxidants" work as "pro-oxidants" in combination with ATO in tumor cells, but not normal cells. Clearly, ROS is an important mediator of the ATO-induced cell death signal, however, the complex responsible for ROS production in response to ATO is controversial. We investigated the role for NADPH oxidase (NOX), an ROS-generating complex, in ATO-induced apoptosis using both chemical and genetic inhibition of the enzyme. ATO-induced death was not affected by the NOX

inhibitor apocyanin at concentrations where NOX is inhibited in a lucigenin assay. This was confirmed in PLB-985 cells, which were deleted of the NOX component gp91. GP91-/- cells were as sensitive to ATO-induced ROS production and apoptosis as the wild-type, indicating NOX has no role in the cytotoxicity of ATO. Next, we investigated the role of mitochondria in ROS production post-ATO treatment. We found that ATO does induce mitochondrial ROS as assessed by flow cytometric analysis with MitoSoxRed, which specifically detects mitochondrial superoxide. In addition, using mitochondria-specific antioxidants, MitoQ and MnTmPYP, we see that inhibition of mitochondrial ROS protects NB4 cells from ATO-induced apoptosis. These results suggest that mitochondria are the targets for ATO-induced ROS and this ROS results in initiation of the apoptotic signaling cascade.

2123 EVIDENCE OF AUTOIMMUNE-RELATED EFFECTS OF TRICHLOROETHYLENE IN STUDIES IN MICE AND HUMANS.

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EPA's IRIS Program is preparing a health assessment for trichloroethyelene (TCE). Many studies of potential autoimmune-mediated effects of TCE have been conducted in the past decade. We examined these studies to determine the concordance between human and animal data of specific clinical and immunological features, and to examine the strength and consistency across studies of the association between trichloroethylene and systemic autoimmune diseases. Studies conducted in the MRL +/+ mouse report an accelerated autoimmune response in relation to exposure to TCE or metabolites dichloroacetyl chloride or trichloroacetaldehyde hydrate. Effects were seen at 4 weeks exposure to TCE doses as low as 0.1 mg/kg/day in drinking water, and included increased antinuclear antibodies, total immunoglobulin levels, increased secretion of interferon-γ and decreased secretion of interleukin-4, consistent with an inflammatory response. With exposures of 32-40 weeks, autoimmune hepatitis, inflammatory skin lesions and alopecia were seen. Two studies in humans reported an increase in pro-inflammatory cytokines (interleukin-2, interferon-γ) and a decrease in interleukin-4 in occupationally exposed workers or in environmentally-exposed children. Occupational exposure to trichloroethylene has also been associated with a severe, generalized skin disorder that is often accompanied by systemic effects including hepatitis. Nine case-control studies provide data about occupational trichloroethylene exposure (5 for systemic sclerosis, 2 for rheumatoid arthritis, and 1 each for undifferentiated connective tissue disease and small vessel vasculitis). The prevalence of exposure is considerably lower in women compared with men. In men, the studies generally reported odds ratios between 2.0 and 8.0, and in women, the odds ratios were between 1.0 and 2.0. The consistency among the human studies and the concordance between the studies in mice and humans, strongly support an etiologic role of TCE in autoimmune disease. (These views are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA)

2124 ENHANCED INDUCIBLE NITRIC OXIDE SYNTHASE EXPRESSION AND NITROTYROSINE FORMATION IN TRICHLOROETHENE-MEDIATED AUTOIMMUNE RESPONSE.

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There is increasing evidence that nitric oxide (NO) may be involved in the pathogenesis of autoimmune diseases (ADs). We hypothesize that NO overproduction [inducible nitric oxide synthase (iNOS)-mediated] results in an oxidative modification of proteins, which may contribute to the induction of an autoimmune response. To achieve this, groups of autoimmune-prone female MRL +/+ mice were treated with trichloroethene (TCE, 10 mmol/kg, i.p., every 4th day), an environmental contaminant known to induce an autoimmune response, for 6 or 12 weeks. TCE treatment led to higher induction of iNOS in the sera at both 6 and 12 weeks, but the increases were greater at 12 weeks. iNOS mRNA and protein expression were also up-regulated in the livers and kidneys following TCE treatment at both 6 and 12 weeks. Furthermore, nitrotyrosine (NT) levels, to evaluate the post-translational modification of proteins by NO, increased significantly in the sera, livers and kidneys of TCE-treated mice compared to controls, and showed a pattern similar to iNOS induction. The increase in TCE-induced nitrosative stress was associated with significant increases in serum autoantibodies (anti-nuclear- and anti-dsDNA-antibodies). Our results suggest that nitrosative stress is associated with induction/exacerbation of an autoimmune response, and necessitate further studies to establish protein nitration as a pathogenic mechanism in ADs. Supported by NIH ES013510.

2125 EVIDENCE FOR POSTNATAL AUTOIMMUNE DISEASE FOLLOWING DEVELOPMENTAL EXPOSURE TO TCDD IN 24 WEEK-0LD C57BL/6 MICE.

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Recent studies have shown that immune development is altered following perinatal exposure to the environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Furthermore, the effects from such exposure may be more dramatic, persisting into adulthood. The extent to which perinatal TCDD exposure predisposes or enhances postnatal autoimmune disease is unknown. To begin to address this, we conducted a study to evaluate the influence of perinatal TCDD exposure on 24 week-old offspring of high affinity *AhR* C57BL/6 mice. Time-pregnant C57BL/6 (10 dams/treatment) were gavaged on gestational day (GD) 12 with a single dose of 0, 2.5, 5.0 or 10 μg/kg. Examination of the B cell phenotypes at 24 weeks showed a significant decrease of B cell lymphopoiesis in the bone marrow accompanied by differentiation shifting toward marginal zone B cells in the spleen. Autoantibody anti-ssDNA, anti-dsDNA, anti- cardiolipin titers and IgG and C3 deposition in the kidney glomeruli both showed increasing trends toward autoimmunity in the TCDD-treated mice, reaching significance in high dose treatment groups in both genders in anti-dsDNA titers. Female treatment groups showed changes in the thymic parameters and phenotypic expression of CD4 and CD8 markers in thymocytes when compared to controls. Examination of the splenic T cell phenotypes showed that perinatal exposure to TCDD caused marked increase in the percentages and total cellularity of autoreactive Vβ3+ and Vβ17a+ T cells in C57BL/6 spleen. These results suggest that perinatal exposure to TCDD plays an important role in immune dysregulation and postnatal expression of autoimmunity.

2126 PERINATAL TCDD EXPOSURE MODULATES B CELL DEVELOPMENT IN JUVENILE C57BL/6 MICE.

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TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin, or dioxin) is a ubiquitous industrial byproduct and well-known toxic environmental contaminant and has been recently linked to autoimmune disease. In laboratory rodents, hyperproteinuria and immune complex deposition in the kidneys are classic indicators of autoimmunity, suggesting aberrant function in B cell-mediated immunity. Recently in our laboratory, we observed decreased splenic B-cell cellularity and altered migration of the activated B cells in 24 week-old C57BL/6 perinatally exposed to TCCD. This suggested that B-cell maturation in the bone marrow might be altered earlier in life, indicating the need for further study of B-cell maturation in younger mice. In this study, we examined the effects of perinatal TCDD on the stages of B cell development from the bone marrow into the spleen to help determine the adult onset of autoimmunity. Bone marrow and spleen samples were collected from 8-10 week-old C57BL/6 mice exposed to TCDD on gestation day 12. Bone marrow-derived hematopoetic stem cells and splenic B-cells were harvested and enumerated. B-cell phenotypes were analyzed via flow cytometry, with antibodies to pan-B-cell surface marker B220, marker CD24, B cell progenitor marker CD24 and AA4.1 and IgM. Our hypothesis was that alterations in bone marrow lymphopoiesis would be observed in the 8-10 week-old C57BL/6 mice, and that these alterations in bone marrow might be most dramatic in the splenic B-cell compartment. Interestingly, the total percentage of bone marrow B-cell populations (B220+) were significantly increased mainly due to increased immature B cell populations (B220low), while there were significant alterations in the phenotypes of developing B-cells in the spleen characterized by shifting differentiation mainly toward marginal zone in males and transitional 1 in females.Thus in this prefatory study, the results show that perinatal TCDD exposure can dysregulate B cell development in young mice.

2127 EXACERBATION OF POSTNATAL AUTOIMMUNE DISEASE IN 24 WEEK-0LD SNF1 LUPUS NEPHRITIS MICE PERINATALLY EXPOSED TO TCDD.

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Studies in our laboratory have shown that perinatal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) modulates immune development in an immunologically sensitive high affinity *AhR* murine strain. In addition, this immune modulation by TCDD appears persistent and shows evidence of increased incidence of autoimmune disease in adulthood. The degree to which perinatal TCDD exposure

can exacerbate postnatal autoimmune disease in an autoimmune predisposed population has not been shown. To begin to address this, we conducted a study to evaluate the influence of perinatal TCDD exposure in 24 week-old offspring of low *AhR* affinity autoimmune-prone NZB X SWR (SNF1) mice. Time-pregnant NZB X SWR mice (8 dams/treatment) were gavaged on gestational day (GD) 12 with a single dose of 0, 40 or 80 μg/kg. The effects of perinatal TCDD exposure on B cell lymphopoiesis in bone marrow and B cell differentiation in spleen were examined. Cytokine production by stimulated splenocytes was also evaluated. In addition, thymic T cell and peripheral T cell phenotypic expression were characterized via flow cytometry. Female TCDD treatment groups showed changes in the thymic parameters and phenotypic expression of CD4 and CD8 markers compared to controls. Anti-ssDNA, anti-dsDNA, anti- cardiolipin titers and IgG and C3 deposition in the kidney glomeruli were elevated providing evidence of increased autoimmune changes in the perinatal TCDD-treated mice. These preliminary results suggest that perinatal TCDD exposure does accelerate autoimmune disease in a lupus-nephritis murine strain and T cells are a primary target.

2128 EFFECTS OF FEED BORNE FUSARIUM MYCOTOXINS ON INTESTINE AND IMMUNE RESPONSE OF BIRDS INFECTED WITH COCCIDIA.

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The potential for Fusarium mycotoxins to modulate immunity in birds was studied in chickens fed naturally contaminated feed after hatching and infected with coccidia at 8 weeks of age. A polymeric glucomannan mycotoxin adsorbent (GMA, Mycosorb®) was also evaluated. Day-old chicks were randomly divided into 3 groups, 40 birds each and fed diets (1) control (2) contaminated grains or (3) contaminated grains + GMA. Contaminated diets contained up to 3.8 ppm deoxynivalenol (DON), 0.3 ppm 15-acetyl DON and 0.2 ppm zearalenone. Half of the birds in each group were orally inoculated at 8 weeks of age with a standardized mixture of Eimeria acervulina, E. maxima and E. tenella. Samples were collected prior to inoculation, 7 days post inoculation (end of challenge period), and 14 days post inoculation (end of recovery period). Serum IgA levels in coccidia infected birds fed contaminated diet were found to be significantly reduced at the end of recovery period. Flow cytometry of isolated blood lymphocytes revealed that CD8+ cell populations in the same birds were significantly lower than controls. Using real-time PCR, IFN-γ gene expression was found to be up-regulated at the end of challenge period and IL-10 gene expression down-regulated at the end of recovery period in birds on contaminated diets. Villus height (VH) of the duodenum of birds fed the contaminated diets was significantly lower than that of the controls. VH of the jejunum and ileum in the same birds was significantly higher compared to controls, suggesting a compensatory mechanism. It was concluded that Fusarium mycotoxins alter the morphology and function of the intestine as well as the immune response to enteric infections. GMA reduced most of these effects.

2129 TCDD PREVENTS DIABETES IN NON-OBESE DIABETIC (NOD) MICE.

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While 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced aryl-hydrocarbon receptor (AhR) activation has traditionally been associated with toxicity, there is growing evidence suggesting that AhR activation could have therapeutic value for the treatment of diverse diseases. Type I diabetes is characterized by self-reactive T cells that destroy insulin-producing cells in pancreatic islets (PI). The onset of diabetes is associated with a decline in regulatory T cells (Tregs) that otherwise prevent pathogenic T cells from destroying PI cells. Since TCDD-induced AhR activation has been shown to induce Treg-like cells in other models, we hypothesized that TCDD may prevent diabetes by inducing Tregs. NOD mice were treated with vehicle or TCDD from 7 to 31 weeks of age. At 23 weeks of age, half of the TCDD-treated mice were switched to vehicle to determine the persistence of TCDD effects on disease progression. Blood sugar was monitored weekly. At the termination of the study, pancreata were analyzed for T cell infiltrates. Pancreatic lymph nodes (PLN) were harvested and analyzed by flow cytometry to determine T cell phenotypes. By 31 weeks of age the incidence of diabetes was 77% in vehicle- and 0% in TCDD-treated mice. In TCDD-treated mice that were switched to vehicle, 50% developed diabetes in 8 weeks. TCDD treatment profoundly reduced PI infiltration and increased the frequency of Tregs in PLN. Treg frequency returned to vehicle levels in mice that were switched from TCDD to vehicle. Taken together these data suggest that AhR activation may represent a novel avenue for type I diabetes therapy.

2130 GENISTEIN PROTECTS FEMALE NONOBESE DIABETIC (NOD) MICE FROM DEVELOPING TYPE 1 DIABETES.

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Type 1 diabetes is a significant health concern with a global increase in incidence of 3% per year. In addition to genetic factors, environmental triggers are also involved in the development of type 1 diabetes. Among the environmental xenobiotics, endocrine active compounds such as genistein (GEN) may have the potential to modulate the development of type 1 diabetes. GEN is a major isoflavone in most soy products, and is consumed in supraphysiologic levels as nutritional supplements and pharmaceuticals. The objective of this study was to determine if GEN had the potential to change the time of onset and/or the incidence of type 1 diabetes in NOD mice when administered by gavage (0.1 ml/10 g body weight) once every day for 180 days. Five groups of mice (24 animals/group; 6-7 weeks of age) were included: naïve control, vehicle control (25 mM Na_2CO_3), and three GEN treatment groups (2 mg/kg, 6 mg/kg and 20 mg/kg body). Mice were maintained on a soy- and alfalfa-free diet (5K96) during the study, and were monitored for blood glucose changes every week. When compared to the vehicle control, exposure to GEN at 2 mg/kg produced significant decreases ranging from 55% to 80% in the incidences of diabetes (blood glucose ≥ 250 mg/dl) and severe diabetes (blood glucose ≥ 400 mg/dl) starting at week 13 of the study. Significant decreases in the incidences of diabetes were observed starting at week 17 and 15 for the GEN dose groups of 6 mg/kg and 20 mg/kg, respectively; significant decreases in the incidences of severe diabetes were observed starting at week 17 and 16 for the GEN dose groups of 6 mg/kg and 20 mg/kg, respectively. This protective effect of GEN was absent when female NOD mice were maintained on the NIH-07 rodent diet that contained 120 g/kg soybean meal and 40 g/kg alfalfa meal. Overall, treatment with GEN did not significantly affect body weight. Taken together, oral exposure to GEN protected female NOD mice from developing type 1 diabetes when fed a soy- and alfalfa-free diet (Supported by NO1-ES05454).

2131 EFFECTS OF DEOXYNIVALENOL ON THE ANTIBODY FORMATION AGAINST PARVO VIRUS VACCCINATION IN MALE MICE.

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Deoxynivalenol(DON) is a trichothecene mycotoxin produced by Fusarium fungi and contaminated widly in food and feed in the northern temperature regions. DON often induces feed refusal, vomiting and diarrhea, which lead to subsequent losses of weight gain in livestock animals. DON has been reported to induce immune dysfunctions and consequently decrease of disease resisitance. The objective of this study was to demonstrate the influence of low level of DON on immune function especially on the antibody formation by vaccination of Parvo virus in mice. 6 wks old C3H male mice were exposed to low dose of deoxynivalenol (2 ppm in drinking water) for 36 days. On the day 8 of DON exposure, animals were innoculated with 0.5ml of inactivated Parvo virus vaccine intraperitoneally. Mice were sacrificed 24 hrs after the termination of DON exposure and the effects of DON on organ weight and immunological parameters (antibody titer and immunoglobulins (Igs) and cytokine contents) were evaluated. Weight of spleen was decreased by the administration of DON but increased by the inoculation of Parvo virus vaccine with DON administration. Antibody titer by Parvo virus vaccine was suppressed apparently by DON. Serum Ig E level was significantly increased by Parvo virus vaccination but no difference between vaccine and vaccine+DON groups. IL-1α, IL-5, IL-9 and G-CSF of serum cytokines were increased by vaccine+DON group compared to vehicle control group. In thymus, GM-CSF, KC, MCP-1 and Rantes were increased at the treatment groups of DON, vaccine or DON+vaccine but IL-6, IL-9, IL-13 were decreased only by the treatment of DON+vaccine. IL-1α in lymph node and MIP-1β in spleen were increased by the administration of DON compared to control. These result suggest that exposures to low dose of DON suppress immune function, especially the ability of antibody formation against vaccination, mainly via T cells mediated immune fuction.

2132 THE ROLE OF HMGB1 IN THE PATHOGENESIS OF CYSTIC FIBROSIS.

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Cystic fibrosis (CF) is one of the most common lethal autosomal recessive inherited disorders. It is characterized by mutations in the gene encoding the CF transmembrane conductance regulator (CFTR) protein in airway epithelial cells. This disease

often results in chronic pulmonary infection with Pseudomonas aeruginosa, the major cause of morbidity, and mortality in cystic fibrosis patients. CF patients exhibit enhanced production of proinflammatory cytokines, including tumor necrosis factor-alpha (TNF-alpha), interleukin (IL)-1beta, IL-6 and IL-8. Here we show increased levels of high mobility group box 1 (HMGB1) in the bronchoalveolar lavage fluid (BALF) from CF patients and CFTR knockout (KO) mice using western blot analysis. HMGB1, a nuclear protein, is an important mediator of systemic inflammatory diseases. However, its role in CF remains to be elucidated. We previously demonstrated the importance of HMGB1 in inducing the inflammatory response and the impairment of Pseudomonas aeruginosa phagocytosis by macrophages. HMGB1 is markedly elevated in CF mice in response to LPS. Moreover, RAW 264.7 cells incubated with the BAL of CF patients and CFTR-KO mice for 24h lead to significantly impairment in the ability of macrophages to phagocytose and migrate, compared to that with BAL of normal human or wild type mice. This impairment in macrophage functions correlates with increased levels of HMGB1 in conditioned medium. These results suggest that HMGB1 plays a crucial role in the pathogenesis of CF lung disease and identifies HMGB1 as a potential therapeutic target for the treatment of CF patients.

2133 SYSTEMIC AND LOCAL IMMUNOMODULATORY EFFECTS FOLLOWING A SINGLE INTRATRACHEAL EXPOSURE OF 1, 2:5, 6-DIBENZANTHRACENE (DBA) IN ADULT FEMALE B6C3F1 MICE.

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DBA is formed as a result of incomplete combustion of carbonaceous material, and human exposure occurs via inhalation from forest fires, cigarette smoke, and asphalt paving. DBA, a more potent congener of the prototypic polycyclic aromatic hydrocarbon (PAH) benzo(a)pyrene, was administered as a single intratracheal aspiration at doses of 0.3, 3.0, and 30 mg/kg. Previous studies from our lab demonstrated that systemic immunosuppression occurred after 28 days of exposure by subcutaneous injection. The current studies were conducted to evaluate both systemic and local immune effects following intratracheal administration of DBA, a more relevant route of human exposure to PAHs. No effects were observed in the lipopolysaccharide (LPS) or $F(ab')_2$ antibody fragment stimulated B cell proliferation assays. Similarly, no effects were observed in polyclonal IgM antibody production, however a statistically significant reduction in IgG (58%) was observed in spleen cell culture supernatants stimulated 24 hrs with LPS. Additional systemic effects observed were significant immunosuppression in the Delayed Type Hypersensitivity Response and in the IgM plaque assay to sheep red blood cells. Effects were only observed at doses greater than 0.3 mg/kg DBA. Local effects, using bronchoalveolar lavage fluid extracted 24 hours after a single intratracheal exposure of DBA, demonstrated a statistically significant dose responsive increase in the pro-inflammatory cytokines IL-1 and IL-6, and an increasing trend in TNF-α. In addition, increasing trends in IFN-γ, IL-5, and IL-10 were observed, while IL-4 production was not effected. These studies demonstrate that a single administration of DBA produces both systemic immunosuppression and local immunomodulation when exposure occurs via the intratracheal route, simulating the route by which human exposure occurs.

2134 VALIDATION OF AN IMMUNOTOXICITY SCREENING METHOD USING TETANUS TOXOID (TT) IN THE CYNOMOLGUS MONKEY.

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Standard testing for immune function usually comprises of measuring the antibody response to a T-cell dependent antigen (e.g. KLH). However when testing immunomodulatory biologics such as monoclonal antibodies, that target certain components of the immune system, there is the potential to induce anergy or clonal deletion to KLH when co-administered. Assessment of immunotoxicity when either is possible, may lead to results that suggest the animal is immunosuppressed, but is only unresponsive to KLH. In such cases a control antigen such as tetanus toxoid (TT), which is given to the animal prior to treatment with the biologic, may provide a clearer picture of the state of the immune system before and after treatment. In this study we set out to establish an immunisation protocol that results in a robust antibody response to TT in the cynomolgus monkey.
TT was administered twice by intramuscular (i.m.) injection, 7 days apart, at a dose level of 75 IU/dose/animal. Control animals received 2 i.m. injections of PBS also 7 days apart. Serum samples were collected prior to initial treatment with TT on Day 1 and on Day 8 (prior to second injection of TT), 14 and 15, to assess the optimum time point for observing elevated IgG levels in serum. The presence of anti-TT antibodies, in serum, was measured using an ELISA method, developed and validated at Aptuit.
After a single injection of TT a significant increase in anti-TT antibodies was observed in all immunised animals on Day 8, when compared to relevant controls (> 6 fold increase in OD compared to a BSA control). A second injection of TT on Day 8 resulted in a marked increase in specific antibodies, on Day 14 and 15 in 1 of 4 animals immunised. In the remaining 3 animals the antibody titre was similar to the levels observed on Day 8.
A single administration of 75 I.U. of TT via the i.m. route, is sufficient in order to generate a significant specific antibody response in the cynomolgus monkey, and provides a suitable positive control when assessing immune function as part of a pre-clinical safety assessment.

2135 ELECTROSPUN NANO-FIBROUS TISSUE ENGINEERING SCAFFOLDS OF POLYDIOXANONE BLENDED WITH ELASTIN: A STUDY OF IN VITRO IMMUNOMODULATORY EFFECTS.

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Electrospinning is a process that can be used to manufacture non-woven nano-fibrous tissue engineering scaffolds for uses such as vascular replacement and wound dressings. Investigations in our laboratory with electrospun blends of polydioxanone and soluble bovine elastin (PDO-ELAS) have demonstrated significant potential uses, however, minimal evaluations of the interactions between these materials and the host immune system have been conducted previously. This study investigates the immunotoxic effects of electrospun PDO-ELAS following *in vitro* co-culture with splenocytes from female B6C3F1 mice. Endpoints examined include: innate (natural killer cell activity), cell-mediated (anti-CD3 antibody-mediated or concanavalin A-stimulated T-cell proliferation, mixed leukocyte response, and cytotoxic T-cell activity), and humoral immunity (lipopolysaccahride-stimulated or $F(ab')_2$ antibody fragment-mediated B-cell proliferation and the Mishell-Dutton T-dependent antibody-forming cell response to sheep erythrocytes). Results indicate that blends of these materials are immunomodulatory *in vitro*. To date, the Mishell-Dutton assay, which evaluates potential adverse effects on multiple cell types including T-cells, B-cells, and antigen presenting cells such as macrophages, has shown the greatest suppression. Results indicate that all blends of PDO-ELAS produce greater immunosuppression than either PDO or ELAS alone, and some blends, for example 40:60 PDO:ELAS by vol., produce suppression as great as 68% when compared to control cultures. These results suggest that more comprehensive testing than is routinely conducted on the immunomodulatory effects of biomaterials is essential prior to pursuing the use of such materials in man.

2136 IMMUNOLOGICAL ASSESSMENTS IN ONE-YEAR INTRAVENOUS TOXICITY STUDY IN MONKEYS WITH ABATACEPT, A SELECTIVE COSTIMULATION MODULATOR.

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To assess the immunomodulatory activity of abatacept, immune parameters were incorporated into a chronic toxicity study in which cynomolgus monkeys (5/sex/group) were administered abatacept IV at doses of 0 (saline controls), 10, 22, or 50 mg/kg once weekly for 52 weeks. Peripheral-blood lymphocyte phenotyping, abatacept-specific antibodies, serum immunoglobulins (IgG, IgM, and IgA), histamine, complement C3a, tumor necrosis factor -alpha (TNF α), and interleukin-6 (IL 6) levels were assessed at various intervals. After an 8 week recovery period, 2 monkeys/sex/group were used to assess a T-cell-dependent antigen response following immunization with keyhole-limpet hemocyanin (KLH). Abatacept was well tolerated at human exposure multiples of 2, 4, and 9, respectively, with no remarkable changes in peripheral-blood lymphocytes representing T-cell subsets (CD3+, CD3+CD4+, or CD3+CD8+), B cells (CD20+, CD20+CD40+), or natural killer cells (CD3-CD16+). In males at 50 mg/kg, a minimal, transient decrease in serum IgG concentration was observed at week 32 but not at week 51. No drug-related effects were observed on histamine, complement C3a, TNF-α, or IL-6 levels. Abatacept was not immunogenic during the study with the exception of 1 male at 10 mg/kg that seroconverted at Week 39. Although viral screening indicated previous exposure in all monkeys to one or more viruses (Rhesus Lymphocryptovirus, Herpesvirus Simiae B-virus, Rhesus Cytomegalovirus, and Simian Papovavirus), there were no associated clinical manifestations or neoplastic changes. Functional activity of the immune system was demonstrated at all doses by a robust antibody response to KLH following immunization after an 8-week dose-free period.

2137 CHARACTERIZATION OF THE LONG TERM PROINFLAMMATORY EFFECTS OF 2'MOE GAPMER PHOSPHOROTHIOATE OLIGONUCLEOTIDES.

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Although generally less proinflammatory (PI) than phosphorothioate oligonucleotides, long-term studies with several antisense oligonucleotides (ASO) containing 2'-O-methoxyethylribose (2'MOE) modification have shown a gradual multiorgan lymphohistiocytic infiltration. This finding appears to be murine specific as these infiltrations have not been observed in long-term monkey studies. The receptor mediating the PI effects of 2'MOE ASO in mice is unknown as the TLR9 pathway was previously excluded. We sought to further characterize the PI properties of 104838, an ASO that has PI properties representative of most 2'MOE ASO regardless of their targets, and control ASO 141923 with little to no PI effects in mice at 30 and 100 mg/kg/wk (s.c.) for 6 months. Treatment with 104838 resulted in a gradual increase in infiltrates after 9, 17 and 25 weeks. After 25 weeks, 104838 caused mild hepatocytic eosinophilic degeneration with severe sinusoidal inflammatory cells infiltration, while moderate proximal tubular epithelial cells vacuolation and granulation reflected the accumulation of the 104838 in the kidneys. Subcutaneous injection of 104838 treatment caused subcutaneous granuloma in the skin at the injection sites with marked accumulation of basophilic granules in macrophages. The spleen also displayed white pulp hypotrophy, red pulp expansion with extramedullary hematopoiesis, megakaryocytosis, histiocytosis and macrophage infiltration. In contrast, no changes were observed with 141923. Gene profiling was performed on livers collected after 9, 17 and 25 weeks. A number of genes associated with interferon and antiviral responses including Stat1 and MCP-1 were upregulated. Interestingly administration of 104838 to Stat1 deficient mice prevented the induction of those genes up to 72 hours post treatment. Furthermore, plasma MCP-1 levels were not increased in Stat1 KO mice following 104838 treatment. In conclusion, high doses for long periods produced increase in infiltrates that correlated to increase MCP-1 and appeared to be Stat1 dependent.

2138 IMMUNOTOXICITY ASSESSMENT OF YOUNG AND ADULT C57BL/6 MICE FED TRIBUTYLTIN CONTAMINATED FISH IN THEIR DIET.

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Tributyltin compounds, including bis (tri-n-butyltin) oxide (TBTO) and tributyltin chloride (TBTC), are widely used as industrial and agricultural biocides. Relatively high levels of TBT have been detected in some marine organisms. The immune system is the main target of its toxicity. The aim of this study is to evaluate the most sensitive age for the immunotoxic effect of TBT. We examined these effects on lymphoid organ cells by in vivo experiments. Two different generations of male and female C57BL/6 mice were fed 30 days with lyophilised fish contaminated with a single low environmentally relevant dose of TBTC (400 µg TBTC/kg diet). Five immunological parameters were used to asses TBTC immunotoxicity: lymphoid organs weight, lymphoproliferative response, phagocytosis functional essay (PFA), natural killers (NK) cell activity and primary antibody plaque-forming cells (PFC) response. The results for mitogenic assay, phagocytosis and NK cell activity are reported here. Results show that lymphoproliferative response to Concanavalin A (ConA) and lipopolysaccharide (LPS) were altered in young mice exposed to TBT. Significant increases in the phagocytosis activity ($p<0.001$) was detected in exposed young mice for both sexes. NK cytotoxic activity was significantly increased ($p<0.05$) for male mice at the 20: 1 effector: target cell ratio. Thus, these results suggest that the environmentally relevant dose of TBT in fish affect B lymphocytes and T lymphocytes as well as the number of cells involved in the host immunosurveillance mechanism especially in young mice. These results could be useful for risk assessment in human populations consuming fish contaminated with TBT.

2139 IMMUNOLOGICAL ALTERATIONS IN B6C3F1 MICE FOLLOWING ORAL EXPOSURE TO A BROMINATED FLAME RETARDENT MIXTURE: DE-71.

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Polybrominated diphenyl ethers (PBDEs) are one of five major classes of currently used brominated flame retardants (BFRs). This study examined the effects of the commercial PentaBDE mixture DE-71 in adult female B6C3F1 mice on complete blood counts, serum chemistry, thyroid hormones, and traditional immunotoxicity endpoints (proliferation, NK cell activity, T-cell and B-cell immunophenotypes). Mice were exposed via oral gavage for 28 consecutive days. DE-71 was dissolved in DMSO and added to canola oil at 10%. Mice were exposed to a daily total dose of 0, 0.5, 5, 50, or 100 mg/kg (0, 0.017, 0.17, 1.7, 3.6 mg/kg/day). Control mice received 10% DMSO in canola oil. . No changes in body or organ weight were observed. Serum T3 and T4 levels were not altered by treatment. Monocytes were decreased in the 0.5, 5, and 50 mg/kg treatments but not at the 100 mg/kg. T- and B-cell proliferation were both increased compared to control at the 100 mg/kg treatment. NK cell activity was decreased compared to control at the 100 mg/kg treatment. No alterations were noted in thymic T-cell populations. Numbers of CD19+, CD21+, and MHCII+ cells in the spleen were not affected. Numbers of splenic CD4-/CD8- cells were increased over control in the 100 mg/kg treatment while no other changes were seen in CD4/CD8 populations.

2140 EVALUATION OF THE POTENTIAL EFFECTS OF ATRAZINE AND ITS METABOLITES ON THE IMMUNE SYSTEM ROUTINE TOXICITY STUDIES CONDUCTED IN RAT, MOUSE AND DOG.

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The purpose of this work is to evaluate the potential effects of atrazine and its metabolites on the immune system based upon tier I, routine toxicity studies. We reviewed all relevant studies conducted by Syngenta on atrazine and it chloro- and hydroxy metabolites. Overall, the tier I studies, including subchronic and chronic studies conducted in the rat and dog, the lifetime studies in rats and mice, and special in utero/lactation/chronic test and multi-generation studies in rats with atrazine and its metabolites, showed no evidence of immune system effects up to and including maximum tolerated doses. In instances where doses were in excess of the maximum tolerated dose, as indicated by reductions in body weight gain of more than 15% or actual body weight loss, secondary effects on thymus and spleen weights were noted. In addition, high doses appear to affect hematopoietic activity in bone marrow, which is primarily reflected as reduced erythroid and myloid parameters and in extreme cases by the activation of extramedullary hematopoiesis. These effects are considered to be the result of excessively high doses. Furthermore, long-term studies in mice, rats and dogs, did not provide any evidence of impaired immune system function (e.g. intercurrent disease, reduced survival, or immune system tumor prevalence). Therefore, the studies support that atrazine and its metabolites do not adversely affect immune function in intact animals up to and including maximum tolerated doses.

2141 CHARACTERIZATION OF IMMUNE AND ASTHMATIC RESPONSES IN CYNOMOLGUS MACAQUES FOLLOWING ANTAGONISM OF IL-12/23 ACTIVITY WITH USTEKINUMAB (CNTO 1275).

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Ustekinumab is a human antibody that neutralizes the bioactivity of human and non-human primate IL-12 and IL-23. It is currently being studied in clinical trials for the treatment of psoriasis. To identify potential adverse effects of ustekinumab on the immune system, immune responses were evaluated in repeated dose toxicity studies following weekly IV administration of ustekinumab at doses of 9 and 45 mg/kg for up to one month and twice weekly SC dosing at 22.5 and 45 mg/kg for up to 6-months. No adverse effects on humoral response to a neoantigen; proliferative responses to T-cell mitogens, circulating lymphocyte subpopulations or distribution of T- and B-lymphocytes in lymphoid tissue were observed. IL-12 can antagonize immune responses mediated through Th2 T cell lineages which are thought to contribute to allergy and asthma. Therefore, asthmatic responses in sensitized macaques following aerosol antigen challenge with Ascaris suum were evaluated following treatment with ustekinumab. IV administration of single and repeated doses of ustekinumab at doses as high as 45 mg/kg prior to antigen challenge did not exacerbate the asthmatic responses of sensitized macaques. The high dose in toxicity studies, 45 mg/kg, is approximately 45-fold higher than the highest equivalent dose intended to be administered to psoriasis patients. The average Cmax value following the last dose at 45 mg/kg in the SC chronic toxicity study in cynomolgus macaques was 118-fold higher than the median Cmax value of ustekinumab following 4 weekly 90 mg SC doses in patients.

These data suggest that high doses of ustekinumab are not immunosuppressive in cynomolgus macaques; do not exacerbate the severity of Th2-mediated responses in an asthma model and that ustekinumab has a broad therapeutic window with respect to immunotoxicity.

2142 IMMUNOTOXIC EFFECTS OF TRIBUTYLTIN ON SPLENOCYTES IN F1 RATS AFTER SUBACUTE ADMINISTRATION.

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Immunotoxicity is the major effect of tributyltin (TBT) compounds. The effect on the next generation of F1 rats exposed to TBT via the placenta and their dams' milk may be stronger than that on adults. Pregnant Wistar rats were exposed to TBT at 0, 25, 50 and 125 ppm in their food. The exposure was stopped 3 weeks after delivery. Males were sacrificed at 1, 2, 3 or 6 weeks after birth. Splenic lymphocyte populations were analyzed by flow cytometry with cell surface marker proteins: CD3/FITC, CD4, CD8, B220 and NKR-PIA. The rats' mean body weight was significantly lower compared with the control in the 125-ppm group at week 1, all exposed groups at weeks 2 and 3, and the 50- and 125-ppm groups at week 6. Mean relative spleen weight was significantly lower in all exposed groups at week 2, and the 25-ppm at weeks 3 and 6. The mean CD3/FITC (%) was significantly lower in the 125-ppm group at week 1, and in all the exposed groups at week 6. The mean CD3/BE (%) in all the exposed groups was significantly lower at week 6. However, the mean B220 (%) was significantly higher in the 25- and 50-ppm groups at week 2 and all exposed groups at week 6, and the mean NKR-PIA (%) in the 50- and 125-ppm groups was significantly higher at week 2. There were no significant differences in any indexes among the dams. This suggested stronger toxic effect of TBT on the body weight and spleen in F1 rats compared with their dams. More immunotoxic effect of TBT on splenocytes was observed after cessation of the exposure.

2143 SIMULTANEOUS TIME COURSE AND DOSE RESPONSE CHARACTERIZATION OF 2, 3, 7, 8-TETRACHLORODIBENZO-PARA-DIOXIN EFFECTS ON THE *IN VIVO* LPS-ACTIVATED PRIMARY IGM RESPONSE IN FEMALE C57BL6 MICE.

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The humoral immune response, particularly the primary IgM response, is very sensitive to impairment by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). In vivo, ex vivo, and in vitro studies have demonstrated that the B lymphocyte is a sensitive cell type that is directly affected by TCDD, which results in disruption of the primary IgM response. It has been hypothesized that TCDD causes a dysregulation of the differentiation program that guides mature B lymphocytes to antibody-secreting plasma cells. To address this hypothesis a simultaneous time- and dose-response evaluation of the effects of TCDD on lipopolysacchride (LPS)-activated plasma cell differentiation was conducted. Female C57BL6 mice were treated by oral gavage with vehicle (sesame oil) or TCDD (3, 10, or 30 μg/kg), and 4 days following TCDD administration given a single intraperitoneal injection of 25 μg LPS or vehicle (PBS). Single cell suspensions of splenocytes were prepared on days 4, 5, 6, and 7 post-TCDD exposure, from which cell surface marker, gene, and protein expression were assessed. TCDD caused significant reduction in the LPS-activated antibody forming cell response, as much as 70% in the 30 μg/kg treatment. LPS-activated generation of plasma cells was significantly inhibited by TCDD treatment, as evidenced by a decrease in the number of CD138+ cells, as well as CD19+ cells expressing high levels of immunoglobulin kappa light chain and J-chain. Evidence supports a TCDD-elicited dysregulation of LPS-activated Blimp-1 gene expression and decreases in the number of CD19+ cells expressing high levels of Blimp-1. This data shows that TCDD suppresses the in vivo primary IgM response to LPS, a stimulus that directly activates B lymphocytes, possibly though inhibition of the induction of Blimp-1.
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2144 EVALUATION OF THE EFFECTS OF ANTISENSE OLIGONUCLEOTIDES ON INFLUENZA CLEARANCE AND MACROPHAGE FUNCTION IN MOUSE.

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Proinflammatory effects are common findings associated with administration of antisense oligonucleotides (ASOs) in rodents, and are attributed to cytokine production by activated macrophages (MØ). ASOs are also known to accumulate in MØ. Total immunocompetence in ASO-treated rodents, an influenza virus clearance model was used as a means of evaluating MØ, T and B cell function in a mouse host resistance model. MØ function was examined by measuring both phagocytosis/oxidative burst and clearance of Streptococcus pneumoniae. In the host resistance model, ASO (20-mer with phosphorothioate linkages and 2′-MOE modifications at the termini) was administered subcutaneously for 4 weeks prior to influenza virus infection and 3 weeks post-infection at doses of 20, 50, and 100 mg/kg/week. ASO had no effect on body and lung weights. However there was a dose-dependent increase in relative spleen weight (~1.2 -fold over control) after a total of 7 weeks of treatment. A slight reduction in influenza-specific IgG was noted in mice treated with 100 mg/kg/wk ASO, but viral clearance was not delayed or impaired by treatment with ASO. In the absence of viral infection, 4-week treatment of satellite mice with ASO had no effect on total IgG/M or serum amyloid A, but caused a dose-dependent increase in MCP-1. Thus, ASO had no effect on immune function in this host resistance model since there was no effect on viral clearance. In a separate experiment, ASO was administered to mice on Days 1, 3, 5 and 8 via i.p. injection at a dose of 30 mg/kg and peritoneal macrophages were isolated and assayed for activity. Treatment of mice with the ASO had no effect on MØ function, as measured by phagocytosis and respiratory burst activity. Thus, ASO had no adverse effect on the functional activity of peritoneal MØ

2145 PHENOTYPIC CHARACTERIZATION OF LYMPHOCYTE SUBPOPULATIONS IN THE MINIPIG.

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The increased use of the minipig for regulatory toxicology over recent years has highlighted the need to develop methods for the detection of immunotoxicology in this species. The aim of this study was to refine the methods of phenotypic characterization of lymphocyte subpopulations using flow cytometry and to generate background control data for use in toxicology studies in the minipig. Samples of peripheral blood, thymus and spleen were taken from 2-year old Göttingen minipigs (Ellegaard). Three methods of cell separation were evaluated: FACSLysing and Pharmalyse (BD), Optilyse (Beckman Coulter) and standard Ficoll (Pharmacia). Membrane staining was performed using antibodies targeting specific lymphocyte subpopulations: leukocytes (CD45 from Serotec - clone K252-1E4, CD45RA from VMRD - clone PG167A), pan T cells (CD3 from BD - clone BB23-8E6-8C8), Th cells (CD4a from BD - clone 74-12-4), Tc cells (CD8a from BD - clone 76-2-11), γδ T cells (dt T from BD - clone MAC320), and B cells (CD21 from BD - clone B-ly-4, CD79a intracytoplasmic from Dako - clone HM57). The selected clones were either pig-specific or human/primate antibodies that cross-reacted in the minipig. The results obtained for the leukocyte and lymphocyte subpopulations were reproducible in all three organs and were consistent with published data. Further work is, however, required to refine the methods of B cell subset characterisation. This study demonstrates the feasibility of lymphocyte subpopulation characterisation during the course of a routine regulatory toxicology study in the minipig.

2146 VALIDATION OF METHOD FOR DETERMINATION OF ANTI-DA-3803 ANTIBODIES IN RAT SERUM USING ENZYME LINKED IMMUNOSORBENT ASSAY.

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To assess the immunogenic profiles of the newly engineered recombinant human chorionic gonadotropin, DA-3803, validation of ELISA for determination of antibodies against DA-3803 in rat sera was performed. An indirect ELISA was developed and optimized in consideration of parameters such as sensitivity, minimum required dilution, and negative cut off ahead of validation process. Polyclonal anti-DA-3803 immunoglobulin G antibodies purified from the sera of DA-3803 treated rats by affinity purification were used as positive control antibodies. Rat blank sera from 20 individual lots were analyzed for determination of negative cut off and cut point factor. The % CV for intra-assay and inter-assay precision were analyzed with positive control samples and ranged from 0.7 to 8.5 and 15.3 to 21.4, respectively. The sensitivity of this assay was 100 ng/ml at which the OD value was greater than the normalized negative cut off value. Immunodepletion

assay was developed for further confirmation of the positive samples during the in-study analysis. Drug interference was observed from 40 ng/ml of DA-3803 at low positive control, and from 120 ng/ml of DA-3803 at high positive control. Differences between positive controls spiked in PBS and rat blank sera were within ±25% except for one blank serum, which met the acceptance criteria for recovery. Results from stability assessment indicated that anti-DA-3803 antibodies in rat serum were stable at least for 6 hours at room temperature, for 4 weeks in a deep freezer, and during five freeze and thaw cycles. In conclusion, we have developed a method for assessment of immunogenicity elicited by DA-3803 treatment in rats by analyzing the anti-DA-3803 IgG antibodies using ELISA method. All the results met the preset acceptance criteria suggesting that this method is suitable to analyze anti-DA-3803 antibodies in rat serum.

2147 DEVELOPMENT OF A SOLID PHASE IN VITRO CYTOKINE RELEASE ASSAY USING A CUSTOMIZED SUPERAGONIST MONOCLONAL ANTIBODY.

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Administration of therapeutic monoclonal antibodies (mAbs) may result in adverse events such as fever, chills, nausea, headache, chest tightness and hypotension. Such symptoms have been correlated with an increase in serum levels of several pro-inflammatory cytokines. Six healthy volunteers received 0.1 mg/kg of TGN1412, a humanized superagonist anti-CD28 IgG4, and subsequently developed multi-organ failure, which was at least partially attributed to a "cytokine storm". Thus, the need to develop an *in vitro* assay to evaluate the safety risks for biotherapeutics is vitally important to ensure the safety of clinical subjects. A customized anti-CD28 superagonist was generated from published sources (IgG4, IgG2, and IgG1 subclasses; termed s-MAb G4/G2/G1) to evaluate in our *in vitro* human whole blood (HWB) cytokine release assay (CRA). When the soluble IgG4 form was tested at 1, 10, and 100 μg/ml no cytokine release above background was detected, similar to previously reported results. It was suggested that simple ligation of CD28 was insufficient for cytokine release and that cross-linking of TGN1412 or the involvement of other cells types (endothelium) might be required. Of the various methods evaluated dry-coating multi-well plates with s-mAb and subsequent incubation with human peripheral blood mononuclear cells (PBMCs) resulted in a significant increase in cytokine release. We demonstrated that s-MAbs can behave as costimulators of T cell activation and also stimulate cytokine production in the absence of T cell receptor signaling. Purified human lymphocytes also produced cytokines in response to s-MAb exposure. Current efforts are focused on validating the assay for predicting cytokine release using known positives and negatives. Following validation, the assay's utility to improve the human risk assessment of therapeutic mAbs that modulate immune function or target immune cells will be evaluated.

2148 DEVELOPMENT OF *IN VITRO* MODEL TO ASSESS PROINFLAMMATORY PROPERTIES OF 2'-MOE OLIGONUCLEOTIDES.

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Second generation antisense oligonucleotides (ASO) with 2'-O-methoxyethlyribose (2'MOE) gapmer design show a significant reduction in proinflammatory (PI) responses compared with first generation ASO. Even so, lymphohistiocytic infiltrations are still observed in liver after long-term treatment with some 2'MOE ASO suggesting that they trigger a mild PI response in rodents (histiocytic infiltration, elevated MCP-1 levels, upregulated interferon stimulated genes, ISGs). We sought to better understand the nature of the PI effects and determine if a specific cell type contributes to the PI response observed with long-term 2'MOE ASO treatments. In particular, we aimed to determine whether liver sinusoidal endothelial cells (LSEC), resident or infiltrating immune cells caused early PI effects. Several in vitro models were developed to test the PI potential of 2'MOE ASO. Murine non-parenchymal cells (NPC) were isolated by liver perfusion. NPCs were cultured for 5 days in media containing epidermal growth factor to favor the expansion of LSEC prior to treatments. Primary bone marrow derived (BDMC) myeloid dendritic or plasmacytoid dendritic cells were also generated by adding either GM-CSF or Flt-3. After 7 days, the different populations were treated with ASOs or positive controls such as LPS and poly(I:C). In addition, the different populations were also characterized by flow cytometry to confirm their identities. After 6, 12 and 24 hours after treatment, the supernatants were collected for cytokine profiling and RNA was isolated for gene expression profiling.
The various populations maintained characteristic phenotypes. GM-CSF BDMCs were the most responsive to the LPS and Poly(I:C) than Flt3 BDMCs and displayed a marked upregulation of IFN-β and MCP-1 mRNAs as well as other ISGs. In contrast, 2'MOE ASOs had little to no effects on the expression of those genes. 2'MOE ASO have been shown to cause gradual infiltration in long term studies. However, those effects cannot be easily extrapolated to an in vitro culture systems thereby supporting the mild PI nature of the 2'MOE ASOs

2149 T-CELL DEPENDENT ANTIBODY RESPONSE IN CYNOMOLGUS MONKEYS FOR IMMUNOTOXICITY EVALUATION.

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[Purpose] It is recommended in ICH Harmonised Tripartite Guideline (S8) "Immunotoxicity Studies for Human Pharmaceuticals" that, in the absence of a specific target, an immune function study such as the T-cell Dependent Antibody Response (TDAR) should be conducted. In the present study, we evaluated immunosuppressive action of cyclosporin (CsA) by TDAR assay using Keyhole Limpet Hemocyanin (KLH) as the immunizing antigen in accordance with the guideline. [Method] CsA at 0 (control), 12.5, and 25 mg/kg was administered orally twice a day (gavage) to 3 male and 3 female cynomolgus monkeys (3 to 8 years old) for 4 weeks. KLH was administered subcutaneously (without adjuvant) once (2 mg/kg) on Day 10. KLH-specific antibodies (serum IgM and IgG) were determined by ELISA on Days 10, 14, 17, 21, 24, and 28. [Result] In the 25 and 50 mg/kg/day groups, when compared with the control group, dose-dependent suppression or a tendency toward dose-dependent suppression in KLH-specific antibody production was noted from Day 17. Suppression of IgG production by CsA was marked compared with that of IgM. Vomiting, soft stool, or diarrhea, considered to be side effects of CsA, was observed in males and females in the 50 mg/kg/day group from Week 2 of dosing. No abnormalities were noted in body weight or food consumption. [Conclusion] KLH-specific antibody production was suppressed by CsA (50 mg/kg/day). It is thought that TDAR assay using KLH is suitable for evaluation of effects of investigational new drugs on the immune system in monkeys.

2150 ANALYTICAL VALIDATION OF SPLEEN, THYMUS, AND MESENTERIC AND MANDIBULAR LYMPH NODE IMMUNOPHENOTYPING FOR CYNOMOLGUS MONKEY.

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The purpose of this study was to develop and validate tissue immunophenotyping procedures for cynomolgus monkey spleen, thymus, and mesenteric and mandibular lymph nodes. These procedures can and have been used to evaluate changes in lymphoid tissue cell populations and were especially important when changes in lymphocyte populations were not detected in peripheral blood. The accuracy, precision, stability, and linearity of methods developed to measure total T cells (CD3+), T helper cells (CD3+4+), cytotoxic T cells (CD3+8+), B cells (CD3+20+), natural killer cells (CD3-16+) in spleen and both lymph nodes and an array of T cell subtypes in the thymus (combinations of CD3±, CD4±, and CD8±) by flow cytometry were assessed. Three to four 1 cm3 sections of tissue were collected from each animal to evaluate all the study parameters. The assay was linear for all cell concentrations tested (10, 1, and 0.1 million input cells). Three separate tissue samples/organ were compared to determine accuracy and four replicate tests/sample were tested for precision. The assay was accurate and precise for spleen and thymus evaluation with most coefficient of variation values less than 10%. As expected, the lymph node analyses yielded much greater variability, likely due to structural differences within each tissue portion. The assay was accurate for T and B cell measurements in mandibular lymph nodes and T cell measurements in mesenteric lymph nodes and precise for both lymph nodes for T and B cells (coefficient of variation values of less than 10%). Results of stability testing showed all stained samples of fixed single-cell suspensions were stable for up to 72 hours and that whole tissue samples were stable for up to 24 hours. In conclusion, tissue immunophenotyping methods have been validated and accurately and precisely measure lymphocyte populations in the spleen and thymus. Methods for mandibular and mesenteric lymph nodes are validated for T cell populations, but may not precisely measure B cells (mesenteric lymph node) or natural killer cell populations.

2151 EVALUATION OF CIRCULATING IMMUNE COMPLEXES (CIC) BIOASSAY™ ELISA KIT TO DETERMINE THE SAFETY PROFILE OF MONOCLONAL ANTIBODIES IN MICE.

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Interaction of antigen to antibody can form immune complex (IC) locally in the extravascular fluids or in the circulation. Even though the ICs are removed harmlessly by the reticuloendothelial system, high level of ICs can cause activation of inflammatory mediator pathways such as the complement system, which in turn leads to disease.

Elevated levels of CICs is involved in glomerulitis, autoimmune diseases, neoplastic diseases, infectious diseases due to bacteria, viruses (hepatitis, mononucleosis), parasites and many other diseases.
The CIC BioAssay™ ELISA kit utilizes the binding property of CIC to C1q. The presence or absence of CIC in mouse serum is determined by comparing with the absorbance of positive and negative controls. Unknowns were incubated in C1q coated plates. After a wash, anti-mouse IgG-HRP is added, followed by another wash. Then, TMB substrate is added for color development and the reaction is stopped. Absorbance is read at 450nm.
The positive presence of CIC in sample is calculated by first subtracting the absorbance of the blank values from those of controls and unknowns. Then, the absorbance of negative control is multiplied by 2. An unknown is reported as positive for the presence of CIC, if it is greater than two fold of the absorbance of the negative control.
In our study, twenty naïve mice serum samples were treated with vehicle and different concentrations of antibodies and then evaluated with BioAssays™ CIC ELISA Kit. Negative control values were less than 0.300 OD and positive control values were all greater than 0.800 OD, meeting the QC criteria set forth by the manufacturer. The %CV for all the controls and unknowns between the replicate wells were < 25%.
In the animals treated with vehicle, 4/5 mice in tested negative for CIC and in the treated groups, 4/5 mice had positive results for the presence of CIC as hypothesized. In summary, The CIC BioAssay™ ELISA kit is suitable for detecting pathogenic CIC as a biomarker for nonclinical safety studies in mice.

2152 IMMUNOPHENOTYPING OF CD4 T LYMPHOCYTES FROM WHLOLE BLOOD OF CYNOMOLGUS MONKEYS BY FLOW CYTOMETRY IS IMPROVED BY USAGE OF CLONE L200.

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Weak staining of the CD4 antibody clone M-T477, even when conjugated to the bright fluorophore phycoerythrin, led to difficult analysis by flow cytometry. Herein we describe detection of CD4 positive T helper lymphocytes by using Clone L200. When incubated at a final concentration of 273 ng/mL, a portion of the CD3+ T lymphocytes within whole blood from Cynomolgus Monkey stained positively with a mean fluorescent intensity (mfi) of 37. In contrast the M-T477 antibody required a much higher concentration of 4.5 ug/mL to achieve a mfi of only 11.7. Comparison with their respective isotype control antibodies at equal concentrations determined a signal to noise ratio of 114 for clone L200 versus 13 for M-T477, yielding a 1 log improvement in binding intensity. An inter sample precision assay resulted in a %CV of 2.85 for CD4 T lymphocytes, similar to the average 3.12 for all tested lymphocyte subsets and mononuclear cells. Inter animal variability was high (11.7 %CV) relative to intra animal variability over time (3.96%), supporting the role of genetic variability as the largest influence on assay precision in Cynomolgus Monkeys. Robustness assays determined minimal effects between analysts, instruments, and red blood cell lysis contamination, and stability assays determined blood stability of 48 hours but only when stored at ambient temperature. In conclusion, optimal antibody characteristics are important when analyzing samples which are being treated with drugs which target or could otherwise modulate immune cell populations and surface receptor expression. Clone L200 was tested with a panel of antibodies to detect B lymphocytes, T lymphocytes, NK cells, and monocytes for binding under a variety of conditions and determined to be fit for the purpose for whole blood immunophenotyping from Cynomolgus Monkey.

2153 OPTIMIZATION AND KINETICS OF THE PRIMARY AND SECONDARY T CELL DEPENDENT ANTIBODY RESPONSE MODEL IN WISTAR RATS.

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The T-cell dependent antibody response (TDAR) is a common assay used to evaluate immunotoxicity potential of compounds in preclinical research. However, the sensitivity of the assay and it's ability to detect a slight immunosuppression have been questioned. This study was thus undertaken with a two-fold objective: 1) Find an optimal route of administration for KLH. 2) Find an optimal dose of KLH that would increase the level of antibody response. Three doses of a known immunosuppressive agent, FK506, were also used in order to evaluate the ability to detect a slight immunosuppression. Wistar rats were dosed once daily for 28 or 32 days either with vehicle, 0.5 mg/kg/day FK506, 1.5 mg/kg/day FK506 or 3.0 mg/kg/day FK506. Immunization with Keyhole Limpet Hemocyanin (KLH) on Days 12 and 20 or 23 enabled the assessment of a primary anti-KLH IgM and IgG response 7, 9 and 11 days post-immunization and of a secondary response 7 and 9 days post-boost. Blood and spleen immunophenotyping was also performed at termination. Immunization was done subcutaneously with 50, 300, 600 or 1000 μg of KLH with or without alum or with 300 μg of KLH intravenously.
The anti-KLH IgM and IgG response was higher and less variable in animals that were immunized via subcutaneous injection when compared to those that received KLH intravenously. Immunosuppressive effects were seen in both sexes and more so in animals that received a high dose of FK506. Notably, an almost complete inhibition of the anti-KLH IgM and IgG response was observed in most of these animals. Striking decreases in relative percentages and absolute counts of CD3+ and CD3+/CD4+ cells were also observed, irrespectively of the KLH route of administration or dose.
Based on the lower variability, almost complete absence of non-responder animals and level of response observed in the TDAR data, and considering the ability to detect immunosuppression, 300 μg KLH administered subcutaneously was considered optimal.

2154 IMMUNOPHENOTYPING OF RAT WHOLE BLOOD USING AUTOMATED LYSIS AND FIXATION LEADS TO IMPROVED PROCESS EFFICIENCY AND PRECISION.

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Under existing ICHS8 guidelines for the assessment of immunotoxicology associated with small chemical drugs it is suggested that flow cytometry may be an effective method to define immune populations. Our previous method involved staining with a pan-leukocyte marker CD45 (clone OX-1) which was unstable during an automated red blood cell lysis and fixation procedure thereby necessitating a manual lysis procedure that consisted of 5 centrifugation steps. Herein we describe a modified method that does not use OX-1, separating cell populations first with forward and side scatter rather than CD45. Cell recovery is enhanced with addition of fetal calf serum prior to fixation and only 1 wash step versus 5 was used, before lysis, which determined to improve the signal to noise ratio of CD3 mean fluorescent intensity 1.9 fold. The modified method was validated by performing 9 assays to test precision, robustness, and stability. LymphSUM values were consistently above 90% indicating specific antibody binding, stable samples, and proper red blood cell lysis. Although stable at ambient temperature, rat whole blood was unstable when stored at 4°C nominal prior to staining and this loss of stability was restricted to CD3 positive T lymphocytes. At 6 hours the CD3 positive T lymphocyte population had decreased by 48%, whereas ambient temperature storage of whole blood prior to processing resulted in only a 6% decrease after 24 hours. In summary, a method has been developed that allows isolation of immune cell populations without relying on OX-1 and therefore allowing usage of automated lysis and fixation. The modified method increased processing efficiency 3 fold due to the reduced numbers of centrifugation steps (1 versus 5), increased red blood cell lysing efficiency, and maintained precision as defined by LymphSum, T lymphocyte subset analysis, and the specific tests described herein.

2155 PARTIAL PHENOTYPIC AND FUNCTIONAL CHARACTERIZATION OF LEUKOCYTES FROM RYR1 R163C MH MICE.

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Ryanodine receptors (RyRs) are ER calcium channels expressed in all nucleated cells. The three RyR isoforms are differentially expressed in cells of hematopoietic origin. RyR1 is preferentially expressed in peripheral monocytes, dendritic cells and B lymphocytes. To understand the role of the RyR1 on the development and function of these cells, we compared bone marrow and splenic myeloid and lymphoid cells from knock in mice expressing the R163C gain-of-function mutation in the RyR1 gene to those from wild type littermates. The R163C mutation predisposes heterozygous human carriers and knockin mice to the disease malignant hyperthermia (MH). Flow cytometry assays revealed differences in the prevalence of murine B cell precursors in R163C bone marrow. In the spleen, R163C mice exhibited significant differences vs wild type littermates in (1) the overall cell number and (2) the proportion of leukocyte subsets. In proliferation assays R163C splenocytes displayed an unexpected pattern of reactivity to mitogens. These findings suggest that RyR1 has had a previously underappreciated role in the development and function of myeloid and lymphoid immune cells.

2156 ROLE OF THE CULLIN 4B, E6AP AND MDM2 UBIQUITIN E3 LIGASE ENZYMES IN LIGAND-DEPENDENT AND INDEPENDENT DEGRADATION OF THE AH RECEPTOR.

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The Ah receptor (AHR) is degraded both in vivo and in vitro following binding to ligands typified by 2,3,7,8-tetrachlorodibenzo[p]dioxin (TCDD). The AHR is also degraded after cells are exposed to compounds such a geldanamycin (GA). Interestingly, the GA and TCDD mediated degradation mechanisms appear to involve different pathways although there is compelling evidence that the degradation event involved in both pathways is mediated via the ubiquitin proteasome pathway (UPP). The connection between a target protein and the UPP is through interaction with the E3 class of ubiquitin enzymes termed ubiquitin ligases. Thus, an siRNA approach was used to reduce the level of E3 enzyme in culture cells so that the level of TCDD and GA induced degradation of the endogenous AHR could be evaluated. Targeted enzymes included: cullin 4b, that has been implicated in TCDD-mediated reduction in ER by the AHR; E6AP, implicated in degradation of various cellular receptors, and MDM2, implicated in control of the p53 tumor suppressor. siRNA specific to cullin 4b was able to reduce the level of endogenous protein by >80% in the Hepa-1 line with no significant impact on the basal concentration of the AHR protein or on the level of TCDD or GA mediated degradation of the AHR. When studies were repeated in the human ARPE-19 retinal epithelial line or HepG2 hepatoma line, reduction in cullin 4b also did not impact the ability of TCDD to induce the degradation of the AHR. For the E6AP enzyme, reduction of the protein by >80% in the Hepa-1 line did not reduce TCDD-induced degradation of the AHR. In addition, AHR degradation was still inhibited by treatment of cells with actinomycin D, suggesting that the denovo mechanism of degradation had not been altered. Finally, the TCDD-mediated degradation of the AHR in the Hepa-1 line was not changed following reduction of the MDM2 protein. These results indicate that none of these well-characterized E3 ligase enzymes appears to be involved in the TCDD or GA induced degradation of the AHR. Supported by ES015481

2157 ROLE OF DNA BINDING AND DIMERIZATION WITH ARNT IN THE LIGAND-INDUCED DEGRADATION OF THE AH RECEPTOR.

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The Ah receptor (AHR) is rapidly degraded both in vivo and in vitro following binding to ligands typified by 2,3,7,8-tetrachlorodibenzo[p]dioxin (TCDD). The AHR is also degraded after cells are exposed to compounds such a geldanamycin (GA). Interestingly, the GA and TCDD mediated degradation mechanisms appear to involve different pathways although there is compelling evidence that the degradation event involved in both pathways is mediated via the ubiquitin proteasome pathway (UPP). Studies were initiated to investigate the connection between DNA binding of the AHR-ARNT complex and the degradation event. Two stable cell lines were created that expressed the b-1 allele of the AHR containing either i) an alanine substitution at arginine 39 (AHRR39A) to ablate DNA binding, or ii) alanine substitutions at leucines 69 and 71 (AHRA6971L) to ablate dimerization with ARNT. Cells stably expressing wild type AHR served as controls. Western blot analysis indicated that the level of AHR expressed in all stable lines was similar and approximated that of wild type Hepa-1 cells. Treatment of cells with TCDD for 6 or 16 h resulted in minimal induction of endogenous CYP1A1 or reporter gene activity. Importantly, TCDD treatment failed to induce high levels of AHR degradation in comparison cells containing wild type AHR. In contrast, treatment of all cells with GA (an hsp90 inhibitor) resulted in high levels of AHR degradation within 2h of exposure. To investigate the role of ARNT in TCDD-induced degradation in cell culture, various lines were treated with siRNA specific to ARNT and then treated with TCDD. Interestingly, reduction in ARNT resulted in reduce levels of AHR degradation, however, the level of ARNT reduction required to produce the results was variable between the different cell lines. Collectively, these studies support the hypothesis that dimerization with ARNT and DNA binding are required to induce ligand-dependent degradation of the AHR. Supported by ES015481

2158 HSP90 DISPLACEMENT DURING LIGAND-DEPENDENT TRANSFORMATION OF THE ARYL HYDROCARBON RECEPTOR.

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The aryl hydrocarbon receptor (AhR) mediates the toxic and biological effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related aromatic hydrocarbons as well as a wide variety of structurally diverse chemicals. In a process termed ligand-dependent AhR transformation, ligand binding results in nuclear translocation of the AhR protein complex and conversion of the AhR into its high affinity DNA form concomitantly with its dimerization with the nuclear Arnt protein. Utilizing in vitro synthesized AhR, Arnt and AhR variants we analyzed the ability of the bHLH, PASA and PASB domains of the AhR to initiate and support AhR transformation. We found that the isolated AhR PASA/B fragment was sufficient for primary events of transformation (i.e. ligand binding, ligand-dependent conformational change, Hsp90 displacement and Arnt dimerization). Studies using GST-AhR fusions with various amount of the AhR PASA and PASB domains suggested exposure of the AhR region N-terminal to the PASB domain (i.e. PASA domain) as a consequence of ligand binding and presumed conformational change in the PASB domain. Ligand-dependent loss of Hsp90 from the AhR occurred only in the presence of Arnt. Formation of an AhR:Arnt:Hsp90 complex in the presence of Hsp90 binding stabilizing agent molybdate, was not only consistent with displacement of Hsp90 by Arnt in their overlapping binding sites in the bHLH and PASB domains during AhR transformation, but suggested sequential nature of this displacement. Demonstration of ligand-dependent displacement of Hsp90 from an AhR lacking the bHLH region suggests that AhR transformation proceeds through ligand-dependent initiation of Hsp90 displacement/Arnt dimerization in the AhR PASA/B domain with subsequent Hsp90 displacement in the bHLH domain and formation of the AhR:Arnt dimer. Not only do these results emphasize the central role of the PASB domain in the AhR transformation process, but they highlight the role of Hsp90 as a key modulator of the activation event in the AhR signal transduction mechanism.
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2159 IDENTIFICATION OF MURINE AHR RESIDUES THAT MAY A PLAY ROLE IN SIGNAL TRANSDUCTION THROUGH PHOSPHORYLATION.

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Responses to dioxin and related pollutants are mediated by the aryl hydrocarbon receptor (AhR) that regulates transcription of target genes. However, the exact mechanism of this process has not yet been determined. Phosphorylation of the AhR/ARNT transcription complex is necessary for its function: phosphorylation of ARNT is required for heterodimerization and AhR phosphorylation is essential for DNA binding and subsequent transcriptional regulation. We attempted to identify amino acid residues that may play a role in signal transduction through their phosphorylation. Using chemical cleavage of AhR isolated from [32P]-orthophosphate-labeled cells, we determined that AhR is highly phosphorylated within amino acids 368-605 and 636-759. These data correlate well with the AhR phosphorylation profile from phosphoprediction algorithms. The highest prediction score for potential sites of phosphorylation are for protein kinases: PKC at T376 and S394, and PKA at S393 and S395. Since phosphorylation of proteins on multiple sites is a common regulatory mechanism in cell signaling, we created a series of AhR mutants with single or multiple mutations in predicted residues and investigated their functional abilities. While DRE-binding potency of AhRs with single or multiple mutations were unchanged, the transcriptional capacities of these mutants were significantly altered. Activation of a DRE-driven reporter gene by AhR mutants was measured in transiently transfected cells and was reduced by 10-25% when AhR contained single mutations. The greatest reduction was observed for mutants T376A and S395A. Transactivation potentials of AhRs with double and triple mutations were decreased by 30-40%. We also demonstrated that both PKC and PKA were able to incorporate 32P into in vitro expressed wild type and mutated AhR proteins. These data support the hypothesis that phosphorylation plays an important role in AhR function by providing an additional regulatory layer in the cell signaling cascade. Supported by NIH Grants ES02515 and ES01247

2160 EPIDERMAL GROWTH FACTOR RECEPTOR PATHWAY BLOCKS ARYL HYDROCARBON RECEPTOR-MEDIATED TRANSCRIPTION AND DIFFERENTIATION IN EPIDERMAL KERATINOCYTES.

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The aryl hydrocarbon receptor (AHR) is a basic helix-loop-helix transcription factor whose activation by ligands such as 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) results in the increase in expression of numerous genes, including the metabolizing enzymes cytochrome P450 (CYP) 1A1 and CYP1B1. While ligand-mediated transactivation of the AHR and transcription of CYP1A1 have been extensively studied, less is known about the cellular regulation of AHR transactivation. We found that in cultures of primary normal human epidermal keratinocytes activation of the epidermal growth factor receptor (EGFR) pathway by its ligands, epi-

dermal growth factor and transforming growth factor-α, opposed the AHR-mediated increase in CYP1A1 and CYP1B1 mRNA and their enzyme activity in a concentration-dependent fashion. Studies of mechanism showed that these effects were not due to changes in CYP1A1 mRNA turnover, the level of AHR protein or a decreased sensitivity of the AHR to ligand activation and nuclear accumulation. While electrophoretic mobility shift assays showed the continued ability of the AHR complex to bind to specific enhancer sequences, nuclear run-on assays identified initiation of transcription as the target of EGFR signaling. As EGFR ligands also prevented the ability of TCDD to accelerate terminal differentiation, and because EGFR ligands could act when given prior to or after TCDD-treatment, the EGFR signaling pathway is identified as a molecular switch that can modify AHR signaling in an autocrine/paracrine manner.

2161 SERINE 395, A SITE IMPORTANT FOR TRANSCRIPTIONAL ACTIVITY IN THE MOUSE ARYL HYDROCARBON RECEPTOR (AHR), IS PHOSPHORYLATED BY PROTEIN KINASE A.

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The aryl hydrocarbon receptor (AhR) is a member of the basic helix-loop-helix transcription factor family. The AhR interacts with a wide variety of xenobiotic agonists, the most potent of which is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), to produce a wide variety of biologic and toxic responses such as teratogenicity and cancer. It has been suggested that AhR activity is regulated by post-translational modifications. Phosphorylation of the AhR, a known phosphoprotein, is a key regulatory mechanism that plays a role in DNA binding and transcriptional activity; however specific sites of phosphorylation have yet to be identified. These studies investigated possible functional phosphorylation at the STS (serine 393, threonine 394, and serine 395) region of the mouse AhR. This sequence is located in a major region of phosphorylation and in the transcriptional inhibitory domain. Phosphorylation prediction algorithms suggest that serine 393 and serine 395 may be phosphorylated by protein kinase A (PKA). We found that PKA is able to phosphorylate the full-length receptor as well as peptides containing the STS sequence via 32P incorporation in vitro. Additionally, Fourier Transform Mass Spectrometry (FTMS) analysis of PKA-treated peptides shows that serine 395 is phosphorylated. Functionally, we show that there is decreased transcriptional activity when serine 395 is mutated to alanine without affecting binding of the AhR-ARNT (aryl hydrocarbon receptor nuclear translocator) complex to dioxin-response elements. These data suggest that phosphorylation of serine 395 regulates the transactivation ability of the mouse AhR. The results from these experiments identify a functionally significant phospho-site and further confirm that the AhR is regulated by phosphorylation. (Funded by NIH Grants ES02515, ES01247, and ES07025.)

2162 *IN VIVO* LEFLUNOMIDE EXPOSURE MIMICS THE EFFECTS OF TCDD IN AN AHR-DEPENDENT MANNER.

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By conducting a search for molecules that lead to increased expression of AHR target genes, we identified leflunomide as a potent aryl hydrocarbon receptor (AHR) agonist. Leflunomide is used to treat rheumatoid arthritis because its primary metabolite (A77 1726) inhibits T cell proliferation by blocking de novo pyrimidine synthesis. We investigated the in vivo effects of leflunomide and its metabolite on AHR-dependent responses in zebrafish to determine if leflunomide could be used as a therapeutic modulator of AHR activity. Previously we reported that inappropriate AHR activation in early life stage zebrafish leads to developmental toxicity, inhibition of fin tissue regeneration, and CYP1A induction. The regeneration endpoint is assessed by surgically amputating the caudal fins of 48 hour post fertilization (hpf) zebrafish; non-chemically exposed animals accomplish complete tissue replacement in three days. Both leflunomide and A77 1726 inhibited tissue regeneration, but only leflunomide induced CYP1A protein expression at concentrations as low as 2.5uM. A77 1726 did not lead to induced CYP1A expression at any concentration tested, suggesting that A77 1726 is not an AHR ligand. To determine if the leflunomide effects were AHR-dependent, the expression of AHR2 was reduced using AHR2 morpholino oligonucleotides. Leflunomide blocked regeneration in control morpholino injected animals, but in the absence of AHR2 expression, leflunomide failed to induce CYP1A expression or block tissue regeneration indicating that these leflunomide-dependent effects are AHR2-dependent. These studies indicate the power of the zebrafish model to mechanistically evaluate chemical biological interactions in vivo, and specifically suggest that at least some of the clinical effects of leflunomide may result from unrecognized AHR activation.

2163 LIGAND-DEPENDENT DIFFERENCES OF DNA BINDING BY AHR-ARNT COMPLEXES *VERSUS* AHR-ARNT2 COMPLEXES *IN VITRO*.

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The aryl hydrocarbon nuclear translocator (ARNT) is a member of the basic-helix-loop-helix PER/ARNT/SIM (bHLH-PAS) family of proteins and functions as a dimerization partner for a variety of bHLH-PAS proteins such as the aryl hydrocarbon nuclear receptor (AHR), hypoxia-inducible factor 1-alpha, and the SIM protein. Importantly, there are several ARNT isoforms and ARNT and ARNT2 exhibit a high level of amino acid identity and appear to dimerize with the same partners. Although knockout of ARNT or ARNT2 is lethal, the mice show different phenotypes, which could be due to the specificity of each ARNT to a given pathway. Previous studies describing the ability of ARNT2 to associate with the AHR in a manner similar to ARNT have shown conflicting results. Interestingly, these studies all employed the low-affinity polycyclic aromatic hydrocarbon AHR ligand, 3-methylcholanthrene. In contrast, current studies have shown that under conditions using the higher-affinity AHR ligand, TCDD, both ARNT and ARNT2 appear to exhibit an equal ability to associate with the AHR and bind DNA when expressed in vitro. Interestingly, studies examining the ability of 3-methylcholanthrene or benzo[a]pyrene activated AHR-ARNT or AHR-ARNT2 complexes to bind DNA showed distinct differences in comparison with studies examining the ability of TCDD activated AHR-ARNT or AHR-ARNT2 complexes to bind DNA. In these studies, when 3-methylcholanthrene or benzo[a]pyrene was used as the activating ligand for the AHR, the resultant AHR-ARNT2 complexes exhibited a greatly reduced ability to bind DNA in comparison to AHR-ARNT complexes activated with TCDD, 3-methylcholanthrene or benzo[a]pyrene, and also exhibited a distinct difference from TCDD activated AHR-ARNT2 complexes. Thus, the ability of AHR-ARNT or AHR-ARNT2 complexes to bind xenobiotic response elements appears to be ligand dependent.

2164 RELATIVE POTENCIES OF INDIVIDUAL DIOXIN-LIKE COMPOUNDS FOR *IN VITRO* CYTOCHROME P450 1A1 TRANSACTIVATION OF ARYL HYDROCARBON RECEPTOR FROM BAIKAL SEAL.

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Our previous study has demonstrated that the expression levels of cytochrome P450 (CYP) 1A1 were positively correlated with the concentrations of dioxins and related compounds (DRCs) including polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and coplanar polychlorinated biphenyls (PCBs) in the liver of wild Baikal seals (Pusa sibirica), indicating that these CYP isozymes are induced by these DRCs. To evaluate the sensitivity to effects of DRCs via Baikal seal aryl hydrocarbon receptor (bsAHR) signaling pathway, and to estimate the relative potencies for induction of CYP1A1 expression by DRCs in Baikal seal, AHR transactivation potency was determined using an in vitro reporter gene assay that bsAHR expression plasmid and a reporter plasmid containing mouse CYP1A1 promoter were transiently transfected into COS-7 cells. The bsAHR was transactivated by the treatment of individual DRC congeners in a dose dependent manner. The EC50 values of these congeners for bsAHR transactivation were at least as high as those for AHR from C57BL/6 mouse, suggesting that bsAHR may be highly sensitive to DRC exposure. Relative potencies (REPs) for transactivation of bsAHR by DRC congeners were estimated as follows; 2,3,7,8-TCDD (1.0) > 2,3,7,8-TCDF (0.041) > 1,2,3,7,8-PeCDD (0.0048) ≈ 2,3,4,7,8-PeCDF (0.0047) ≈ PCB126 (0.0033) > PCB118 (0.000084). As for about 50% of the wild Baikal seal population, the hepatic total TEQs, that were calculated by using the REPs of individual DRCs, exceeded the 2,3,7,8-TCDD-EC50 estimated in this study. These results support our earlier study indicating that the accumulation levels of DRCs in wild Baikal seals reached the levels sufficient to induce CYP1A induction through bsAHR transactivation. Thus, the bsAHR reporter gene assay system can be a valuable tool for evaluating the susceptibility to DRCs in Baikal seal, and further assessing the risk in the wild population.

2165 RESPONSIVENESS OF A *XENOPUS LAEVIS* CELL LINE TO THE ARYL HYDROCARBON RECEPTOR LIGAND 6-FORMYLINDOLO[3, 2-*b*]CARBAZOLE (FICZ).

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Frogs are extremely insensitive to the toxic effects of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and related xenobiotics. Aryl hydrocarbon receptors (AHRs) from *Xenopus laevis* (African clawed frog) bind TCDD with at least 20-fold lower affinity

than mouse AHR^{b-1}, a likely mechanism for the lack of TCDD toxicity. Frog AHRs may nonetheless be highly responsive to structurally distinct compounds, especially putative endogenous ligands that may have important physiological or developmental functions. In this study, we sought to assess the responsiveness of the *X. laevis* AHR pathway to the candidate endogenous ligand FICZ, a tryptophan photoproduct that exhibits high potency in mammalian systems. XLK-WG cells, derived from kidney epithelium, were found to express mRNAs encoding AHR1α, AHR1β, ARNT1, and ARNT2, demonstrating that this cell line expresses all fundamental components of the pathway. Consistent with results from rodent cell lines, cells exposed to FICZ for 3 hours expressed 10- to 100-fold greater quantities of CYP1A6 and 1A7 mRNAs than those exposed to the same concentrations for 24 hours, suggesting that induction is rapid but that the compound is subject to metabolic breakdown. In contrast, TCDD induced a sustained response over the same time period. Following a 3-hr exposure, the EC50 of FICZ for CYP1A7 mRNA induction was ~50 nM, comparable to TCDD but substantially higher than reported for rodent cells. mRNA from cells treated with 100 nM FICZ, 100 nM TCDD, or vehicle was analyzed using Affymetrix GeneChips. This experiment confirmed the extreme low responsiveness of the frog system to TCDD, which induced only 3 transcripts and repressed only one ≥2-fold. However, the list of transcripts similarly altered by FICZ was longer, including 25 induced transcripts and 3 repressed. Overall, these studies demonstrate the responsiveness of XLK-WG cells to FICZ but suggest that like TCDD, its potency is substantially lower than observed in mammalian systems. [NIH R15 ES011130; HHMI Education Grant]

2166 DISTINCT REGULATION OF THE ARYL HYDROCARBON RECEPTOR ACTIVITY BY DIFFERENT LIGANDS.

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The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that is involved in the regulation of adaptive responses to cellular and xenobiotic stress stimuli. AhR activity is highly regulated by ligand binding, but also by negative feedback mechanisms such as depletion of ligands through metabolism, receptor degradation and the action of the AhR repressor (AhRR).
In this study, we used the human hepatoma cell line HepG2 to investigate mechanisms controlling AhR function and activity. AhR signalling was characterized after activation by two groups of high affinity ligands, the metabolizable AhR ligands, 6-formylindolo[3,2-b]carbazole (FICZ) and indolo[3,2-b]carbazole (ICZ), and the non-metabolizable ligand 2,3,7,8-tetrachlorobenzo-p-dioxin (TCDD). We observed temporal differences in gene expression patterns of two AhR target genes, CYP1A1 and AhRR, dependent on the particular ligand used. Both genes showed a transient induction response when activated with FICZ and ICZ, while TCDD treatment resulted in sustained upregulation. Interestingly, we also noted differences in the kinetics of the binding of the activated receptor to the xenobiotic response elements in the CYP1A1 promoter, as analyzed by chromatin immunoprecipitation assays. AhR, when activated by FICZ and ICZ, bound more rapidly to its cognate response elements than the TCDD-activated receptor which, in addition, remained bound to DNA for a longer period of time. Finally, we observed differences in ligand-induced degradation of the AhR. A rapid but transient decrease in total AhR protein levels was observed in cells treated with FICZ and ICZ in contrast to a sustained downregulation following TCDD treatment.
In summary, this study shows that the function of the AhR is highly regulated with regard to receptor stability and transcriptional activation properties by different classes of agonistic ligands. This enables the cell to modulate its response to different stimuli through the AhR pathway.

2167 LIGAND SPECIFICITY IN MODULATING ARYL HYDROCARBON RECEPTOR DNA BINDING.

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The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that mediates many of the toxic and biological responses observed following exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, dioxin) and related compounds. Following ligand binding, the AhR translocates into the nucleus, dimerizes with the AhR nuclear translocator protein (ARNT), and binds to specific DNA recognition sites known as dioxin responsive elements (DREs), stimulating transcription of downstream genes. While the canonical DRE contains an invariant core sequence of 5'-GCGTG-3', our mutagenesis studies have identified key nucleotides adjacent to the core sequence that are required for maximal ligand:AhR:ARNT complex formation and transcriptional activation. While our previous work has shown no ligand-specific differences in the nucleotide specificity of AhR DNA binding, recent studies have suggested that this can occur. Specifically, while the upstream regions of the murine Bax and human Pon-1 genes appear to confer AhR-dependent responsiveness to PAH ligands (dimethylbenz[a]anthracene-3,4-dihydrodiol (DMBA-DHD) and 3-methylcholanthrene (3MC), respectively), but not to TCDD, the induction was suggested to be mediated by a non-DRE sequence. In contrast, our gel retardation analysis revealed that neither DMBA nor 3MC were able to stimulate AhR:ARNT binding to these "nonclassical" DNA response elements. In addition, in transfection experiments, DMBA and TCDD were unable to induce AhR-dependent luciferase gene expression from a reporter plasmid containing the novel Bax "DRE" sequence upstream of the luciferase gene in mouse hepatoma (Hepa1c1c7) cells. Taken together, our results do not support the previously reported ligand-dependent differences in AhR DNA binding specificity. Non-biased approaches to study AhR DNA binding, such as the PCR-assisted binding site selection technique, will be essential to further confirm or disprove whether ligand-specific differences in AhR DNA binding specificity exist. (ES012498, ES007685 and ES007059)

2168 NEWSPAPERS AND NEWSPAPER INK CONTAIN AGONISTS FOR THE AH RECEPTOR.

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The aryl hydrocarbon (AhR) is a promiscuous ligand-dependent transcription factor whose activation by structurally diverse chemicals leads to a variety of toxic and biological effects. High throughput bioassays have been used to further identify and characterize new classes of AhR ligands and to also identify AhR active chemicals in a variety of materials in order to determine the magnitude and extent of human exposure to these chemicals through normal daily activities. Solvent extracts of newspapers obtained from countries throughout the world activate the AhR and stimulated AhR-dependent gene expression in a transient manner. Agonist activity was also identified in solvent extracts from both unprinted virgin paper, and black printing ink, demonstrating the presence of AhR agonists in extracts from both materials. The extracts could compete with [^{3}H]TCDD for AhR binding and also stimulated AhR transformation and DNA binding in vitro. DMSO extracts of printed newspaper could induce CYP1A1-dependent EROD activity in zebrafish embryos in vivo. Bioassay-directed chemical fractionation approaches were carried out to attempt to separate and identify the AhR agonists present in newspaper and printing ink. Multi-step solid phase extraction and reverse-phase HPLC fractionation of sample extracts was carried out and those fractions containing AhR agonist activity were analyzed by GC/MS. While tentative identification of a variety of diverse chemicals present in the active newspaper extracts was obtained, further fractionation and chemical analysis is necessary. While the responsible bioactive chemical(s) and their biological and/or toxicological significance remain to be elucidated, our results demonstrate that newspapers and printing ink contain relatively potent/efficacious and metabolically-labile AhR agonists. Given the quantity of global newspaper recycling and re-processing, the presence of these novel AhR agonists in wastewater streams should be determined and their potential effects on aquatic organisms assessed.

2169 ARYL-HYDROCARBON RECEPTOR (AHR), AGING AND IMMUNITY.

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The endogenous role of AHR is poorly understood. Based on the effects of its most potent ligand, TCDD, we hypothesized that AHR responds to dietary and/or endogenous ligands to down-modulate the immune response and that AHR-deficient mice ($AHR^{-/-}$), over a lifetime, may develop heightened immune responses and increased inflammatory diseases. Thus, identifying pathologies associated with aging in $AHR^{-/-}$ mice may elucidate vital endogenous functions of the AHR. To this end, we compared general pathology, cytokine production and immune cell phenotypes in young adult and 24-month old $AHR^{+/+}$ and $AHR^{-/-}$ male and female mice. Two predominant pathological conditions were noted in aged $AHR^{-/-}$ mice: rectal prolapse and liver tumors. Aged female $AHR^{-/-}$ mice also showed an increased incidence of tonic-like seizures. Supernatants of splenocytes cultured with anti-CD3 and LPS for 24, 48, or 72 hr revealed AHR-dependent changes in IL-6, IL-12, and IFNγ levels. IL-6 was increased in cultures from $AHR^{-/-}$ mice regardless of age and gender. IFNγ levels were increased in cultures from old $AHR^{-/-}$ males only. IL-12 was increased with cells from young female and old male $AHR^{-/-}$ mice. Under Th2-polarizing conditions, IL-5 but not IL-10 levels were elevated with cells from $AHR^{-/-}$ males regardless of age. Phenotype analysis of splenocytes revealed that the normal, age-associated shift in T cell frequency from naïve to memory and regulatory

phenotypes was not influenced by AHR status. However, aged $AHR^{-/-}$ mice had increased frequency of memory $CD8^+$ T cells compared to the aged $AHR^{+/+}$ controls. While aged $AHR^{-/-}$ mice displayed increased frequency of $CD4^+CD25^+$ T cells, expression levels of Foxp3 decreased relative to $AHR^{+/+}$ controls regardless of age. Proliferation of CFSE-labeled CD4, CD8 and B cells cultured with anti-CD3 and LPS was significantly reduced in aged mice of both genders regardless of AHR status. These data suggest that, over a lifetime, AHR may function to suppress tumors and protect against inflammation. Supported by NIEHS grants P01ES00040 and P30ES00020.

2170 ARYL HYDROCARBON RECEPTOR (AHR) REPRESSES ACUTE PHASE RESPONSE GENE EXPRESSION IN A DNA-BINDING INDEPENDENT MANNER.

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In the recent years, there has been a considerable focus on identifying novel mechanisms for transcription factor activity. DNA-binding independent effects have been identified for receptors such as GR and ER. An endpoint for analyzing these effects has been the ability to influence the activity of other transcription factors. We investigated the ability of AhR to function in the absence of direct DNA-binding. A previously characterized DNA-binding mutant (A78D) form of AhR, along with the wild-type (WT) form of AhR, was transiently expressed in Simian virus 40 immortalized AhR-null mouse hepatocytes. Changes in gene expression were analyzed using Affymetrix microarrays. Serum amyloid A3 (Saa3) was one of the genes whose expression was repressed 2-fold with the A78D-form as well as the WT-from of AhR. Saa3 is an acute-phase protein and is significantly induced by pro-inflammatory cytokines, particularly interleukin (IL)-6 and IL-1β. Microarray results were verified with real-time PCR in an independent experiment, which also included two additional mutants of AhR – a heterodimerization and a nuclear localization mutant – neither of which were able to repress Saa3 expression. Subsequent experiments, conducted in Hepa1c1c7 cells, demonstrated that AhR activation with different ligands can suppress IL-mediated Saa3 mRNA expression. Moreover, the fact that TCDD failed to repress Saa3 expression in primary AhR-null hepatocytes demonstrated that the effect is AhR-dependent. Chromatin immunoprecipitation assays revealed that AhR activation diminishes the acetylation of histones as well as recruitment of the p65 component of NFκB at the Saa3 promoter in response to IL. Other acute-phase response genes, notably Saa1 and Saa2, were also observed to be significantly repressed on AhR activation. Our results provide evidence for a DNA-binding independent mode of AhR activity. This study also demonstrates that AhR can modulate the acute-phase response, thus establishing a role for AhR in inflammatory signaling.

2171 COMBINATION OF INFLAMMATORY SIGNALING AND AN AHR LIGAND LEADS TO SYNERGISTIC INDUCTION OF IL-6.

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Research has increasingly shown connections between inflammatory immune responses and carcinogenesis and cancer progression. Evidence points to interleukin 6 (IL-6) differing in its effects by tumor type, in some cancers acting as a positive growth factor, increasing anti-apoptotic signaling, and increasing migration and invasion properties. We are investigating the combinatorial effects of IL-1β and Ah receptor ligands in tumor cell lines and their synergistic induction of IL-6 production. Co-treatment of MCF-7 breast cancer cells and ECC-1 endocervical cancer cells with IL-1β and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) for 2 hours was shown via quantitative RT-PCR to result in a synergistic induction of IL-6 mRNA transcription and a decrease in CYP1A1 mRNA levels. Quantitative RT-PCR and ELISA assay revealed continuous increases in both mRNA and serum protein levels throughout a 72 hour time course of treatment, with no signs of abating. Additional AhR ligands, including benzo[a]pyrene, similarly show synergistic activity in conjunction with IL-1β, with regard to IL-6 induction. Although the promoters for IL-6 and IL-8 have marked similarities in containing NFκB, C/EBPβ and c-Jun regulatory binding sites, IL-8 transcription does not follow the pattern of synergistic increase seen with IL-6. Therefore, the combination of pro-inflammatory IL-1β and AhR ligands shows a context-specific ability to up-regulate cellular IL-6 output for a sustained period of time. This event could lead to instigation of both autocrine and paracrine loop signaling within the tumor microenvironment. These results would suggest a mechanism that could in part explain the tumor promoting properties of certain AhR ligands (e.g benzo[a]pyrene).

2172 USE OF COMPLEMENTARY AND ALTERNATIVE MEDICINES DURING BREAST CANCER TREATMENT – POTENTIAL INTERACTIONS WITH AROMATASE INHIBITORS.

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A major problem for women during breast cancer treatment is the sudden onset of menopause and symptoms related to that, such as hot flashes. Although some treatment is available, many women seek for alternative therapies. These alternative therapies (or CAMs, complementary and alternative medicines) are often plant-based, thus containing bioactive phytochemicals, and can easily be obtained in drug stores and through the internet. Considering the high number of breast cancer patients using CAM (up to 80%), the increasing risk for adverse interactions with breast cancer treatment is of major concern.
A commonly used CAM in relieving menopausal symptoms is the soy phytochemical genistein. Our studies with H295R cells (human adrenocorticocarcinoma cells) showed that genistein can affect aromatase inhibition by fadrozole at a concentration ($2.5x10^{-8}$M) that is comparable with human plasma concentrations after fadrozole treatment. A low concentration of genistein (10^{-7}M) further increased aromatase inhibition by fadrozole in vitro, while a higher concentration of genistein (10^{-5}M) could negate the fadrozole effect and increase aromatase activity with almost 60% compared with fadrozole-treated cells. The genistein medium concentrations tested were within the range of plasma concentrations that can be obtained by a normal diet, but plasma levels can increase dramatically to micromolar levels upon soy-based supplement use. Taken together, these data suggest that phytochemicals that are used by women against menopausal symptoms during breast cancer treatment can potentially affect the efficacy of their treatment. Further studies are ongoing in a recently developed breast cancer model (co-culture of primary mammary fibroblasts and MCF-7 mammary tumor cells) to determine the potential effect of CAM interaction with aromatase inhibitors on tumor cell growth.

2173 4-NEROLIDYLCATHECOL (4NC), A COMPOUND FROM POTHOMORPHE UMBELLATA, AS A CYTOTOXIC AGENT FOR HUMAN METASTATIC MELANOMA CELLS.

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Potential in vitro cytotoxicity antitumoral activity of 4NC and derivatives has been described. However, this effect has never been evaluated in human melanoma cell lines. Here we investigated 4NC effects regarding cytotoxicity and inhibition of invasion of three human metastatic melanoma cell lines (SK-Mel 28; SK-Mel-103 and SK-Mel-147) using matrigel precoated transwell chambers. Cytotoxicity of 4NC was evaluated by trypan blue exclusion (IC50> 10μg.ml -1 for all cell lines). Invasion inhibition in the range of 85-100% using IC10 was observed in comparison to non-treated cells. From these results, it was suggested that molecular targets of 4NC should be explored to contribute to rational design of more effective treatment for this daunting clinical problem.

2174 DECREASED INCIDENCES OF NEOPLASMS AND NON-NEOPLASTIC LESIONS IN F344/N RATS AND B6C3F1 MICE FED MILK THISTLE EXTRACTS FOR TWO YEARS.

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Milk Thistle, Silybum marianum, a member of the Aster family, is a tall edible plant. The fruits of this plant contain flavono-ligans silybin, silydianin and silychristin. Milk thistle has been used as an herbal medicine to treat liver disease. Milk thistle is regulated under the 1994 Dietary Supplement Health and Education Act (DSHEA). Oral herbal medicines, such as milk thistle, are considered a "dietary ingredient" under this act, and often there are few standard toxicity studies reported in the literature. The National Toxicology Program (NTP) tested this compound because of its wide spread human exposure and little information available on its long term toxic effects. Groups of 50 male and 50 female F344/N rats and B6C3F1 mice were given Milk thistle extract in feed at doses of 0, 12500, 25000 or 50000 mg/kg for two years. There was no treatment related death or clinical signs of toxicity in rats or mice of either sex. Dose related decline in the body weights were noted in the rats (two highest dose groups) and mice (highest dose group) of either sex as compared to their respective controls. There was significant dose-related decrease in incidence of mammary gland fibroadenomas in treated female rats. The

incidence of nonneoplastic lesions in liver (leukocytic infiltrates and bile duct hyperplasia), glandular stomach (erosions), pancreas (atrophy) and eye (cataract) was lower in treated rats as compared to control rats. There was a significant dose-related decrease in incidence of liver tumors in male mice. The incidence of some nonneoplastic changes in thyroid (degeneration), liver (foci of altered hepatocytes), and kidney (nephropathy) was also lower in treated mice. These preliminary findings suggest that milk thistle or its components may have antiproliferative and anti-inflammatory properties in mammals that result in a decrease in the development of background nonneoplastic and/or neoplastic lesions.

2175 SAPONINS DERIVED FROM ROOTS OF PLATYCODON GRANDIFLORUM INHIBIT AP-1 TRANSACTIVATION AND CELL TRANSFORMATION IN BEAS-2B HUMAN BRONCHIAL EPITHELIAL CELLS.

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Epidemiological data suggest that consumption of fruits and vegetables has been associated with a lower incidence of cancer. Platycodon grandiflorum is a traditional oriental herbal medicine that is known for its immunostimulatory and antitumor effects. In this study, we investigated effects of Changkil saponins (CKS) derived from roots of Platycodon grandiflorum on phorbol myristate acetate (PMA)-induced activator protein-1 (AP-1) transactivation and cell transformation in BEAS-2B human bronchial epithelial cells. We found that CKS significantly blocked PMA-induced cell transformation in a concentration-dependent manner. CKS significantly attenuated PMA-induced AP-1 activation, a transcription factor. CKS inhibited the PMA-induced phosphorylation of extracellular-signal regulated protein kinases (ERK) and p38kinases, which regulate AP-1 activation. Taken together, our results suggest that inhibition by CKS of PMA-induced neoplastic transformation in BEAS-2B cells is related to blocking of ERK and p38 activation, and also attenuation of AP-1 activation. These results indicated that CKS has potent anti-cancer-promoting activity.

2176 EFFECTS OF THE COFFEE DITERPENE KAHWEOL ON THE INHIBITION OF PROLIFERATION AND INDUCTION OF APOPTOSIS IN HUMAN LUNG ADENOCARCINOMA A549 CELLS.

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Many foods contain non-nutritional constituents that may have beneficial health effects, such as anti-inflammatory and anti-carcinogenic properties. Recent studies have highlighted the chemoprotective effects of kahweol, a coffee-specific diterpene. However, the precise underlying protective mechanisms are poorly understood. To elucidate these mechanisms, we investigated the effects of kahweol on cell cycle progression and induction of apoptosis in human lung adenocarcinoma A549 cells. The data from cell viability assay showed that kahweol exhibited the most potent anti-proliferation effect on A549 cells in a time- and dose-dependent manner. This event is accompanied the arrest of A549 cells at the G0/G1 phase by kahweol. Treatment of kahweol significantly decreased viability of A549 cells due to apoptosis and cell-cycle arrest through down-regulation of Bcl-2 and up-regulation of p21, p27, p53, and Bax. Kahweol also induced the cleavage of caspase-3 and PARP (poly ADP-ribose polymerase). In addition, kahweol-induced apoptosis was confirmed by TUNEL assay. These results demonstrate that kahweol has significant on the inhibition of proliferation and induction of apoptosis.

2177 SAPONINS ISOLATED FROM THE ROOT OF PLATYCODI RADIX INHIBITS THE INVASION AND MIGRATION OF HT1080 HUMAN FIBROSARCOMA CELLS *VIA* THE INHIBITION OF MMP-2 ACTIVATION AND DOWN REGULATION OF MMP-9 AND MT1-MMP.

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Matrix metalloproteinases (MMPs) play important roles in solid tumor invasion and migration. In this study, we showed that saponins isolated from the root of Platycodi Radix (Changkil saponins: CKS) dose-dependently inhibited HT1080 cell invasion and migration, and decreased MMP-2 and MMP-9 activities. Furthermore, CKS reduced MMP-2 expression at protein and mRNA levels, and suppressed 12-O-tetradecanoylphorbol-13-acetate (TPA)-enhanced expression of MT1-MMP without changing tissue inhibitors of metalloproteinase (TIMP)-2 levels. CKS also suppressed TPA-induced increases in MMP-9 protein and mRNA levels, but did not alter TIMP-1 level. Our results suggest that CKS may be a valuable anti-invasive drug candidate for cancer therapy by suppressing MMP-2, MMP-9, and MT1-MMP.

2178 BIS[3'-(5-BROMOINDOLYL)]METHANE INDUCES KRüPPEL-LIKE FACTOR 4-DEPENDENT ACTIVATION OF P21 IN COLON CANCER CELLS EXPRESSING WILD-TYPE OR MUTANT P53.

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Bis(3-indolyl)methane (DIM) is a phytochemical with a broad spectrum of anti-cancer activities. We have developed a series of synthetic symmetrical ring-substituted DIM analogs, including 5,5'-dibromoDIM, which exhibit enhanced activity as inhibitors of cancer cell and tumor growth compared to DIM. In colon cancer cells, 5,5'-dibromoDIM decreased cell survival and inhibited G0/G1 to S phase progression, and this was accompanied by induction of the cyclin-dependent kinase inhibitor p21 in HT-29 and RKO colon cancer cells. Mechanistic studies demonstrated that induction of p21 in both RKO (p53 wild-type) and HT-29 (p53 mutant) cells by 5,5'-dibromoDIM was KLF4-dependent and induction of p53 in RKO cells was also KLF4-dependent. Analysis of the p21 promoter in p53-dependent RKO cells showed that 5,5'-dibromoDIM activated p21 gene expression through the proximal GC-rich sites 1 and 2, and chromatin immunoprecipitation assays showed that KLF4 and p53 bound to this region of the promoter, whereas in HT-29 cells, unidentified upstream cis-elements were required for induction of KLF4. 5,5'-DibromoDIM (30 mg/kg/d) also inhibited tumor growth in athymic nude mice bearing RKO cells as xenografts, demonstrating that ring-substituted DIMs such as 5,5'-dibromoDIM represent a novel class of mechanism-based drugs for clinical treatment of colon cancer.

2179 DIHYDROARTEMISININ SUPPRESSES PMA-MEDIATED MATRIX METALLOPROTEINASE-9 GENE ACTIVATION THROUGH DOWN-REGULATING NUCLEAR FACTOR-KAPPAB AND C-JUN.

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Matrix metalloproteinase-9 (MMP-9) plays an important role in the invasion and metastasis of cancer cells. Dihydroartemisinin (DHA) is a semisynthesized agent from the artemisinin first extracted from the Chinese plant Artemisia annua. Previous studies have shown that artemisinin derivates, apart from their antimalarial activity, possess antitumor, antiangiogenic, and anti-inflammatory effects. In this study, we examined the inhibitory effect of DHA on phorbol myristate acetate (PMA)-induced MMP-9 expression in HT1080 human fibrosarcoma cells. DHA significantly and selectively suppressed PMA-induced MMP-9 expression transcription in HT1080 cells. Furthermore, as evidenced by MMP-9 promoter and electrophoretic mobility shift assays, DHA specifically inhibited MMP-9 gene expression by blocking PMA-stimulated activation of NF-κB and c-Jun. In addition, DHA suppressed PMA-induced phosphorylation of Raf, MEK, JNK and extracellular signal-regulated kinase (ERK), upstream factors involved in NF-κB and c-Jun activation, whereas the phosphorylation of p38 mitogen-activated protein kinase was not affected by DHA, suggesting that the primary target of DHA for suppression of the NF-κB and c-Jun induction is present in upstream of ERK or JNK signaling pathway. These results suggest that the suppression of MMP-9 expression, at least in part, contributes to the antitumor activity of DHA.

2180 EPIGALLOCATECHIN-3-GALLATE IS A NOVEL HSP90 INHIBITOR.

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Numerous animal studies have shown that epigallocatechin-3-gallate (EGCG), a major component of green tea, protects against certain types of cancers, although the mechanism has not yet been determined. Our laboratory demonstrated that EGCG blocks the aryl hydrocarbon Receptor (AhR) -mediated transcriptional ability induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Unlike other AhR

antagonists that directly bind to the AhR, EGCG blocks AhR-mediated transcription by binding to hsp90. The purpose of this investigation was to detail the mechanism whereby EGCG affects hsp90 function. The use of a proteolytic footprint assay indicates that EGCG directly modulates the conformation of hsp90 and binds to a C-terminal ATP binding site of hsp90. Hsp90 chaperone function, as illustrated by refolding of denatured luciferase, is inhibited by EGCG treatment, but is reversed by adding exogenous hsp90. Hsp90 dimerization, which occurs at the C-terminal end, is also inhibited by EGCG treatment. These data suggest that EGCG acts as an hsp90 inhibitor. Coimmunoprecipitation studies show that EGCG stabilizes an AhR complex composed of hsp90 and XAP2, decreases the association of aryl hydrocarbon nuclear translocator (Arnt) to ligand-activated AhR. Thus, EGCG, through its ability to bind to AhR-associated hsp90, alters AhR conformation, although translocating the AhR into the nuclei, decreases AhR's association with Arnt and blocks dioxin response element (DRE) recognition. These studies indicate a novel mechanism whereby EGCG blocks ligand-induced DRE binding and inhibits the AhR-mediated transcription activity by acting as a unique hsp90 inhibitor. Since hsp90 inhibitors are being intensively studied for cancer therapy in clinical trials, this investigation will advance further research that may ultimately lead to the use of EGCG and/or of its derivatives for chemotherapeutic purposes. Funded by NIH Grant ES014364, and Center Grant ES01247.

2181 METHYL 2-CYANO-3, 11-DIOXO-OLEAN-1, 12-DIEN-30-OATE (CDODA-ME) IS HIGHLY CYTOTOXIC TO PANCREATIC CANCER CELLS.

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Glycyrrhetinic acid is a major triterpenoid acid in licorice extracts and this compound has been synthetically modified in the A ring and methylated to give methyl 2-cyano-3,11-dioxo-18β-olean-1,12-dien-30-oate (CDODA-Me). Previous studies showed that CDODA-Me was cytotoxic to colon cancer cells and activated peroxisome proliferator-activated receptor γ (PPARγ). CDODA-Me was also cytotoxic to Panc1 and Panc28 pancreatic cancer cells with growth inhibitory IC_{50} values of 1.10 and 1.95 μM, respectively. CDODA-Me also activated PPARγ-dependent transactivation in pancreatic cancer cells transfected with PPARγ-GAL4 chimera and a receptor construct (pGAL4-luc) containing five GAL4 response elements linked to a luciferase reporter gene. The cytotoxicity of CDODA-Me in pancreatic cancer cells was accompanied by induction of apoptosis, multiple growth inhibitory and proapoptotic genes including the cyclin-dependent kinase inhibitors p21 and p27. Increased expression of nonsteroidal anti-inflammatory drug activated gene-1 (NAG-1) and downregulation of cyclin D1 was also observed. Interestingly, cotreatment of pancreatic cancers with CDODA-Me and PPARγ antagonists showed that induction of these responses was PPARγ-independent. It has been shown that upregulation of NAG-1 by PPARγ agonists was dependent on prior induction of EGR-1. Kinase inhibitor studies show that the activation of NAG-1 by CDODA-Me in Panc28 is completely dependent on the PI3K pathway; however, partial inhibition was also observed using p38 and PKC inhibitors. In contrast, in the Panc1 cancer cell line, NAG-1 induction was completely reversed by p38 inhibitors and only partially reversed by MAPK and PI3K inhibitors.

2182 CURCUMIN INHIBITS BLADDER CANCER CELL GROWTH BY TARGETING THE SPECIFICITY FACTORS BY BOTH PROTEASOME-DEPENDENT AND PROTEASOME-INDEPENDENT MECHANISMS.

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Curcumin (diferuloylmethane) is a phytochemical expressed in various Curcuma species that has been extensively used in traditional medicine. This compound also shows promise as a chemotherapeutic agent for cancer treatment. Curcumin modulates multiple pathways and genes in cancer cell lines, and inhibition of nuclear factor κB (NFκB) has been frequently associated with the growth inhibitory and proapoptotic activity of this compound. In this study, treatment of 253JB-V and KU-7 bladder cancer cells with 5 to 50 μM curcumin decreased cell proliferation, and IC_{50} values for this response were 11.7 and 11.9 μM, respectively. Curcumin (10 - 25 μM) decreased expression of cyclin D1 and also decreased levels of the cyclin-dependent kinase inhibitors p21 and p27 in both cell lines. Within this same concentration range, curcumin also induced apoptosis and decreased expression of survivin, vascular endothelial growth factor (VEGF), and VEGF receptor1 (VEFGR1). Previous studies show that expression of survivin, VEGF and VEGFR1 in cancer cell lines is dependent on specificity protein (Sp) transcription factors, and we therefore investigated the effects of curcumin on these proteins in 253JB-V and KU-7 bladder cancer cells. At concentrations as low as 10 μM, curcumin decreased expression of Sp1, Sp3 and Sp4 proteins and decreased the luciferase activity in the cells transfected with Sp1 and Sp3 promoter constructs in both cell lines. The curcumin-induced effects on Sp proteins were both proteasome-dependent and -independent. These results suggest that one of the underlying mechanisms of action of curcumin in bladder (and other) cancer cell lines is the targeted degradation of Sp transcription factors which results in activation of growth inhibitory and proapoptotic pathways.

2183 GUGGULSTERONE, A CONSTITUENT OF INDIAN AYURVEDIC MEDICINAL PLANT COMMIPHORA MUKUL, INHIBITS ANGIOGENESIS *IN VITRO* AND *IN VIVO*.

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Guggulsterone is a plant sterol derived from the gum resin of Indian Ayurvedic medicinal plant Commiphora mukul that has been used for thousand of years for the treatment of different disorders including bone fracture, arthritis, and inflammation. We have shown previously that guggulsterone inhibits growth of human prostate cancer cells, but not a normal prostate epithelial cell line (PrEC), by causing apoptosis. We now report a novel response to guggulsterone involving inhibition of angiogenesis in vitro and in vivo. Guggulsterone treatment inhibited capillary-like tube formation (in vitro neovascularization) in human umbilical vein endothelial cells (HUVEC) and migration in HUVEC and DU145 human prostate cancer cells in a concentration- and time-dependent manner. The guggulsterone-mediated inhibition of angiogenesis in vitro correlated with suppression of secretion of pro-angiogenic growth factors including vascular endothelial growth factor (VEGF) and granulocyte colony-stimulating factor (G-CSF), down-regulation of VEGF receptor 2 protein level, and inactivation of Akt. The guggulsterone-mediated suppression of DU145 cell migration was intensified by knockdown of VEGF-R2 protein level. Ectopic expression of constitutively active Akt conferred significant protection against guggulsterone-mediated inhibition of migration in DU145 cells. Oral gavage of 3 μmol guggulsterone to male nude mice (five times per week) inhibited in vivo angiogenesis in DU145-Matrigel plug assay as evidenced by a statistically significant decrease in (a) tumor burden, (b) microvessel area (staining for angiogenic markers Factor VIII and CD31), and (c) down-regulation of VEGF-R2 protein expression. In conclusion, the present study reveals that guggulsterone inhibits angiogenesis by suppressing VEGF/VEGF-R2 signaling axis and inactivating Akt kinase. Together, our results provide compelling rationale for further preclinical and clinical investigation of guggulsterone against prostate cancer (supported in part by NCI grants CA115498, CA113363, and CA101753).

2184 MORPHOLINO KNOCKDOWN OF CYP1A EXACERBATES CARDIAC DEFORMITIES IN β-NAPHTHOFLAVONE DOSED *FUNDULUS HETEROCLITUS*.

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Morpholino gene knockdown is a powerful technique for studying the role of specific genes/proteins in the mechanisms of toxicity. It has been widely used in zebrafish (*Danio rerio*) and the African clawed frog (*Xenopus laevis*) to investigate developmental pathways and mechanisms of toxicity, but not in environmental models. In this study, the morpholino knockdown technique was significantly altered from zebrafish methods for use in the Atlantic killifish (*Fundulus heteroclitus*). To validate the method, a morpholino targeting CYP1A was used (CYP1A-mo). Knockdown of CYP1A activity was measured by in ovo EROD (ethoxyresorufin-O-deethylase) assay. For the EROD assay, eggs were dosed with 10 ppb β-naphthoflavone (BNF; AhR agonist, CYP1 inducer) and ethoxyresorufin (CYP1 substrate) at 24 hours post fertilization (hpf). Production of resorufin was determined by fluorescence microscopy at 96 hpf. CYP1A-mo injection resulted in up to 71% reduction of EROD activity compared to non-injected or control morpholino-injected embryos. To further confirm CYP knockdown, embryos were scored for cardiac deformities at 120 hpf. Previously, this lab found in zebrafish that knockdown of CYP1A coupled with BNF dosing resulted in increased cardiac deformities. Furthermore, treatment of killifish with a chemical CYP inhibitor and BNF had the same result. In the current study, CYP1A-mo injection in conjunction with treatment with 10 ppb BNF resulted in a significant increase in the occurrence and severity of cardiac deformities, compared to controls. EROD activity remained significantly decreased in injected larvae as late as 480 hpf. CYP1A-mo function was further validated via transcription/translation and Western blot assays. These results

provide the first use of morpholinos to investigate molecular pathways in an established environmental model. Support from the NIEHS-supported Superfund Basic Research Program at Duke University (P42ES10356) and Boston University (P42ES007381), and Integrated Toxicology Program (T32ES07031).

2185 LIVER TOXICITY AND REGENERATION: THE USE OF A SMALL FISH MODEL – JAPANESE MEDAKA.

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The D-galactosamine (GalN) model for cell proliferation has been established previously in rats and proven useful for studying liver regeneration after acute liver injury and chemical induced liver toxicity. In this experiment, the small fish model, Japanese Medaka, was exposed to 1g GalN/kg and sampled at 9 days post exposure. Three male and three female livers were analyzed for changes in morphology and cellular proliferation index. Distinct morphological changes were observed in the livers exposed to GalN. For example, cytoplasmic changes, disorganization of hepatocytes (not in two-cell thickness), constriction/dilation of blood vessels, and infiltration of inflammatory cells were apparent. The cell proliferation index was measured by anti-proliferating cell nuclear antigen (PCNA) staining. Our study showed that cell proliferation was significantly induced following exposure to GalN for 9 days in both male and female livers ($p \leq 0.05$). Future experiments will analyze the apoptotic index and identify the cells responsible for proliferation. The apoptotic index will be measured using the TUNEL assay. This will indicate the extent of cell death following GalN exposure. In addition, future results from double immunolabeling will identify the proliferating cells as hepatocytes, biliary epithelial cells, or both. Results of these studies will further elucidate the mechanism by which cellular proliferation is occurring. The current results showing that GalN similarly affects morphology and cellular proliferation in the small fish model, Japanese Medaka, support further experimentation towards the use of Medaka as an alternative animal model to study liver regeneration following hepatotoxicity.

2186 A MULTIFACETED, MEDIUM-THROUGHPUT APPROACH USING ZEBRAFISH FOR DETECTING DEVELOPMENTAL NEUROTOXICITY.

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To address the EPA's need to prioritize hundreds to thousands of chemicals for testing, we are developing a rapid, cost-effective *in vivo* screen for developmental neurotoxicity using zebrafish (*Danio rerio*), a small freshwater fish with external fertilization. Zebrafish embryos develop rapidly (3-4 days) in a clear chorion, allowing visual inspection throughout development. The screen consists of four general endpoints: death, teratology, nervous system structure and locomotor activity. All maintenance and testing were conducted in a 96-well microtiter plate format at 26°C. Zebrafish embryos were exposed to a range of ethanol concentrations (0.25% to 4%) from 6 hours to 5-days post fertilization (dpf). At 2dpf the nervous systems of some embryos were examined by whole mount anti-acetylated α-tubulin staining; at 6dpf, additional larvae were assessed for death, terata, and locomotor activity. The LC_{50} at 2dpf was approximately 2.6% ethanol; this value dropped to 1.6% ethanol by day 6. The most common terata were edema, microcephaly, and micropthalmia. Embryos exposed to ethanol concentrations below 0.75% did not show an incidence of teratogenic effects different from control embryos. Whole mount anti-acetylated α-tubulin staining at 2dpf revealed a dose-dependent decrease in retinotectal projections. These effects occurred at ethanol doses well below teratogenic levels. Exposure to ethanol below teratogenic doses also elicited changes in locomotor activity. Collectively, these findings demonstrate the potential utility of this multifaceted screen for developmental neurotoxicity using zebrafish larvae. *This is an abstract of a proposed presentation; the information does not necessarily reflect Agency policy.*

2187 IMPROVED DIFFERENTIAL DISPLAY ANALYSIS BASED ON DEGENERATE OLIGONUCLEOTIDE-PRIMED PCR.

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DNA micorarray is very useful for comprehensive study of gene transcription status affected by toxic substances. However, microarray experiments need detailed molecular information and is very expensive for minor species, which microarray sets are not commercially available. Although there is a variety of fish species throughout the world and important fish species are different for each country, possible impact on various gene is hard to be addressed for those minor species. In this study, we attempted to develop a novel differential display technique based on degenerate oligonucleotide-primed PCR (DOP-PCR), using mRNA database of zebrafish (Danio rerio). We used mono-primers for DOP-PCR, as there were many sets of inverse-repeat sequence adequate for PCR reaction in zebrafish mRNA. DOP-PCR primer consisted of 9-mer primed part linked to 3-mer degenerate part (NNN) and 10-mer linker part. Primed part of primers was designed to obtain about 10 bands for each PCR reaction, using our developed computer program "Mono Search RGU ver.1 mRNA". Several clear bands (100- 1,000 bp) were reproducibly obtained with cDNA from whole internal organs of adult zebrafish. Completely designed sequences were obtained for 23% bands. However, 38% bands showed incomplete primer sequence less than 9-mer (4-8-mer) and 39% bands lacked primer sequence for one side. The same primers designed for zebrafish could be used for reproducible PCR bands with medaka (Oryzias latips) and other 8 fish species, although the number of positive bands seemed relatively fewer. These results suggest the usefulness of DOP-PCR in differential display analysis for comprehensive study of gene transcription status for various fish species. This technique can be applied for non-coding RNA. We are improving the method using DOP-PCR primer with longer primed sequence.

2188 DRUG EFFECTS ON THE LOCOMOTOR ACTIVITY OF LARVAL ZEBRAFISH.

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As part of an effort to develop a rapid *in vivo* screen for EPA's prioritization of toxic chemicals, we have begun to characterize the locomotor activity of zebrafish (*Danio rerio*) larvae and the effects of prototype drugs. Zebrafish larvae (6-7 days post-fertilization) were individually maintained in 96-well microtiter plates at 26°C and under a 14:10 light:dark cycle with lights on at 0830 hr. Drugs included nominal (non-lethal) concentrations of ethanol (0.5 – 2% v/v), *d*-amphetamine SO_4 (0.08 – 40 μM) or cocaine (0.2 – 100 μM). For each drug, all doses and controls were present on each plate. A total of 12 – 32 larvae were exposed to each concentration. Locomotor activity was assessed using a Noldus video activity monitor and Ethovision 3.1 software to track each animal under either visual light or dark (infrared) conditions for up to 90 minutes post-dosing. Each of the three chemicals produced dose-related changes in activity. In general, low concentrations increased activity while higher concentrations decreased activity; similar patterns have been obtained in rodent tests of motor activity. These results indicate that very young zebrafish are sensitive to centrally active drugs, and that drug challenges may be conducted in a convenient, microtiter plate format. *T.D. Irons is supported by the NIH NIGMS Initiative for Maximizing Student Diversity.* *This is an abstract of a proposed presentation; the information does not necessarily reflect Agency policy.*

2189 CHARACTERIZATION OF LOCOMOTOR ACTIVITY OF ZEBRAFISH LARVAE: TEMPORAL VARIABILITY AND PHOTORESPONSE.

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As part of EPA's effort to develop a rapid, *in vivo*, vertebrate screen for toxic chemicals, we have begun research to characterize the locomotor activity of 6-day post-fertilization (dpf) zebrafish (*Danio rerio*) larvae. Larvae were individually housed and tested in 96-well microtiter plates; animals were kept at 26°C using a 14:10 light:dark cycle with lights on at 0830 hr. We used the Noldus tracking system to record locomotor activity (measured as total distance traveled) under two different lighting conditions: light (visible light) and dark (infrared light). First, it was necessary to know the optimal time window for testing during the day (*i.e.*, stable overall baseline levels of activity from hour to hour). This was done by testing larvae at different times during the day under either lighting condition. The most stable period for assessing activity was between 1330 and 1630 hr (or at least 5 hr after the beginning of the light period). Second, we wanted to track the pattern of activity under alternating lighting conditions. To do this, larvae were acclimated in the dark for 5-10 min. Data collection began in visible light, followed by dark (infrared), across several cycles. Locomotor activity was low initially during visible light and then increased over the 10-min period. Switching to dark (infrared) produced a large initial increase in locomotor activity, followed by a gradual reduction across 10-min. Repeated changes in lighting conditions slightly reduced the overall level of activity, but did not alter the patterns of activity in either visible light or dark. The changes in locomotor activity to shifts in lighting conditions are reproducible in larval zebrafish, and may be useful in testing the effects of developmental exposure to toxic chemicals on behavioral transitions and habituation. *This is an abstract of a proposed presentation and does not reflect Agency policy.*

2190 DIFFERENCES IN THE STRUCTURE AND FUNCTION OF FATHEAD MINNOW AND HUMAN ERα: IMPLICATIONS FOR IN VITRO TESTING OF ENDOCRINE DISRUPTING CHEMICALS.

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Mammalian receptors and assay systems are generally used for *in vitro* analysis of endocrine disrupting chemicals (EDC) with the assumption that minor differences in amino acid sequences among species do not translate into significant differences in receptor function. We have found that the fathead minnow estrogen receptor alpha (fERα) differs from the human estrogen receptor alpha (hERα) in terms of both the amino acid sequence of the ligand binding pocket and the affinity of some EDCs for the receptor. The fERα was previously isolated from a cDNA library and sequenced. Chemicals are currently being tested for their affinity to either fERα or hERα in two binding assays to compare results from whole cell versus cell-free binding systems. The fERα had reduced ligand binding at a higher temperature (37°C), whereas, ligand affinity for the hERα was similar at both 37°C and room temperature. A few chemicals displayed notable differences in binding affinities among fERα and hERα. These included the antiestrogen ICI, dibutyl phthalate (DBP), and benzyl butyl phthalate (BBP) (fERα IC50s = 41.6±6.7nM, 18.1±7.3μM, and 8.2±2.1μM, respectively; hERα IC50s = 192.6±23.1nM for ICI and could not be calculated for DPB and BBP due to incomplete binding curves). Confirmatory studies in the cell-free binding system are in progress. If confirmed, these results suggest that studies using mammalian receptors and cell systems for *in vitro* screening and testing of chemicals may not always reflect EDC affinity for receptors among lower vertebrates. Funding was provided by the NCSU/EPA Cooperative Training Program CT833235-01-0. Disclaimer: This is an abstract of a proposed presentation and does not necessarily reflect USEPA policy.

2191 ZEBRAFISH: A TOOL FOR EXAMINING THE EFFECTS OF SUB-ACUTE ALGAL TOXIN EXPOSURE IN THE VERTEBRATE CNS.

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Domoic acid (DA) is a diatom-produced neurotoxin that accumulates in filter-feeding marine shellfish and is subsequently transferred through the food web resulting in a severe neurotoxic illness known as amnesic shellfish poisoning (ASP). Acute signs of ASP include vomiting, diarrhea, seizures, memory loss, coma, and death. Dose-response relationships for acute DA excitotoxicity have been well defined for primate, rodent, and fish model species. However, little is known about the effects of sub-acute DA exposure (levels below those shown to induce overt toxicity). Sub-acute exposure is of particular concern for coastal Tribal communities that subsistence fish on razor clams, a shellfish species known to retain DA for up to a year. Currently, clam dig openings are guided by toxin regulatory limits set by the Washington State Department of Health (WDOH). However, the concern is that people are likely exposed to low levels of DA (≤ 20 ppm the regulatory limit) on a regular basis. In the present study, the zebrafish model was used to identify gene expression effects in the CNS associated with both acute and sub-acute DA exposure. Differential gene expression as evidenced by microarray analysis was observed in the brains of both sub-acute and acute treatments compared to controls. The observed gene expression patterns indicated that sub-acute DA exposure impacted the zebrafish CNS, and also that the mechanisms of DA toxicity may be different under conditions of acute and sub-acute DA exposure. Collectively, the dose-responses of DA-induced behavioral injury coupled with the microarray-generated gene expression data suggest that zebrafish are a useful model for exploring the mechanisms of chronic algal toxin exposure relevant to the vertebrate CNS.

2192 IDENTIFICATION AND COMPARISON OF TWO ISOFORMS OF THE FARNESOID X RECEPTOR ALPHA IN JAPANESE MEDAKA (ORYZIAS LATIPES), A DEVELOPING BILIARY TOXICITY MODEL.

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The Japanese medaka (*Oryzias latipes*), a small freshwater teleost, has a history of usage in liver chronic toxicity studies. A mutant strain of medaka with a transparent body wall is of particular interest for non-invasive, *in vivo* analysis of internal alterations. Since little is known about the molecular biology underlying certain medaka liver functions, the goal of this work is to locate and characterize medaka farnesoid X receptor alpha (medFXRα), a member of the nuclear receptor superfamily that plays a key role in bile acid homeostasis. We have identified two isoforms of medFXRα that differ in the AF1 domain and are the result of alternative splicing. Analysis of the medaka genome located medFXRα1 and it was amplified from liver and intestinal cDNA. 5' RACE isolated the 5' UTR of medFXRα1, and during this process a second isoform of medFXRα, medFXRα2, was identified. Preliminary data shows medFXRα1 is more highly expressed in liver than medFXRα2, while the opposite is true in gut. We have also demonstrated activation of both medFXRα isoforms by bile acids and GW4064 through use of transient transactivation assays with a Gal4DBD-medFXRαLBD chimeric construct. Further research is underway to determine functional differences between medFXRα1 and medFXRα2 through *in vivo* and *in vitro* experimentations. It is believed that medFXRα1 and medFXRα2 may differentially activate gene targets in the presence of an agonist. This work is the first characterization of FXRα in a teleost species and creates molecular underpinnings necessary for understanding medaka biliary system function in normalcy and during toxicity. This work is supported by an EPA STAR Graduate Fellowship, and by funds from the National Center for Research Resources and the National Institutes of Health.

2193 MPTP (1-METHYL 4-PHENYL 1, 2, 3, 6-TETRAHYDROPYRIDINE) INDUCED NEURODEGENERATION IN DASYATIS SABINA (ATLANTIC STINGRAY).

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To investigate the feasibility of elasmobranch fish models in the study of neurodegenerative disorders including Parkinson's and Alzheimer's disease, we demonstrate the effects of MPTP exposure on dopaminergic neurons of the hypothalamus and mesencephalic tegmentum in the stingray D. sabina. In addition, alpha-synuclein and amyloid precursor proteins are shown to be present in brain tissue of this elasmobranch fish. A captured population of D. sabina was housed in isolated, static tank systems. After a minimum 10 day acclimation period, animals were exposed to 10ug/kg MPTP. A separate population was administered saline injection to serve as control. 72 hrs following treatment, animals were sacrificed, and brain tissue was processed for immunohistochemical examination. Tyrosine hydroxylase (TH) antibody was used to identify dopaminergic neuron clusters in the hypothalamus as well as the mesencephalic tegmentum. Results demonstrate dopaminergic neurodegeneration in the hypothalamus and mesencephalic tegmentum of D. sabina following 10ug/kg MPTP exposure. In contrast, controls showed no neuronal loss in either brain region. Antibodies for both alpha-synuclein and amyloid precursor protein determined the presence of these proteins in the stingray brain. The results of MPTP exposure, in conjunction with the presence of key biomarkers used in Alzheimer's and Parkinson's research in mammals, demonstrate the viability of the elasmobranch fish as a potential model for the study of environmentally induced neurodegenerative disease.

2194 ARYL HYDROCARBON RECEPTOR REPRESSOR (AHRR): MECHANISTIC INSIGHTS FROM STUDIES IN MAMMALIAN AND NONMAMMALIAN MODELS.

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The AHRR is a bHLH-PAS protein that is induced by an aryl hydrocarbon receptor (AHR)-dependent mechanism and acts as a transcriptional repressor of AHR function. We are using human and piscine model systems to investigate the role of AHRR in regulating signaling through AHR and other transcription factors. Experiments using transient expression of human or zebrafish AHRRs in mammalian cell lines demonstrate that the mechanism of repression does not involve competition for ARNT. Displacement of AHR from AHR response elements contributes to the repression but is not essential, as indicated by studies employing a non-DNA-binding AHRR variant. AHRR does not associate with AHR in co-immunoprecipitation experiments. To assess the specificity of AHRR, we tested its ability to repress transactivation mediated by the bHLH-PAS protein HIF-1alpha and the nuclear receptors ERalpha and PXR. AHRR repressed HIF-1alpha activation of a HRE-luc reporter construct, but did not affect the ability of ER or PXR to activate transcription through their respective enhancer elements. Two human AHRR variants associated with differences in male fertility (AHRR-Pro185 and AHRR-Ala185) did not differ in their ability to repress AHR signaling. AHRR not only repressed transactivation by TCDD-activated AHR but also de-repressed genes repressed by a constitutively active AHR in human tumor cell lines. AHRR

mRNA was highly expressed in adult human testis and was also expressed in fetal heart, kidney, liver, and muscle. Zebrafish embryos in which expression of AHRRa was knocked down by injection of morpholino anti-sense oligonucleotides exhibited cardiovascular abnormalities in the absence of TCDD exposure and were more sensitive to TCDD-induced toxicity. Together, these results suggest that the AHRR regulates both constitutive and ligand-activated AHR signaling and may contribute to cross-talk between AHR and other factors. [R01ES006272, P42ES007381, F32ES013642]

2195 REDUCED SOX9B EXPRESSION BY TCDD IMPAIRS CRANIOFACIAL CARTILAGE GROWTH IN ZEBRAFISH EMBRYOS.

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Non-genetic factors such as exposure to environmental contaminants can disrupt normal development of the early vertebrate skeleton. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) impairs craniofacial skeletal development across many vertebrate species and its effects are especially prominent in early life stages of fish. Zebrafish embryos exposed to TCDD develop shortened cartilage structures in both the upper and lower jaw. Microarrays were used to characterize the TCDD-induced transcriptional response of the craniofacial cartilage in zebrafish larvae and a critical chondrogenic transcription factor, sox9b, was identified to be the most significantly reduced transcript. Therefore, we hypothesized that reduced sox9b expression caused by TCDD stunts growth of jaw cartilages in zebrafish embryos. Consistent with this hypothesis, AB wild-type embryos injected with graded concentrations of sox9b splice-blocking morpholino exhibited the same TCDD-like phenotype. If TCDD acts by reducing sox9b expression, then heterozygous sox9b mutants should be more sensitized to TCDD exposure than wild-type. To test this hypothesis we used a sox9b deletion mutant line and exposed newly fertilized embryos from heterozygous crosses to graded concentrations of TCDD and examined jaw cartilage development at 96 h post fertilization. Consistent with the idea that TCDD works through decreasing sox9b, TCDD did not induce a more severe phenotype in the homozygous sox9b deletion progeny expressing no sox9b at all. Importantly, half of the offspring were more sensitive to TCDD than wild-type siblings. Genotype screening with PCR of embryos after alcian blue staining confirmed sox9b deletion heterozygous offspring produces more severe malformed jaw cartilages with TCDD. These results suggest that reduced sox9b expression in TCDD-exposed zebrafish embryos contributes to jaw malformation. However, the precise mechanism remains to be elucidated. (Supported by NIH RO1 ES012716 and UW Sea Grant).

2196 CHEMICAL GENETICS TO IDENTIFY MOLECULAR PATHWAYS CONTROLLING TISSUE REGENERATION.

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Zebrafish caudal fin regeneration is an established model to study tissue regeneration. Previously, we have demonstrated that TCDD exposure inhibits caudal fin regeneration and we have utilized TCDD as a chemical probe to understand the molecular pathways important for regeneration disrupted by the activation of AHR pathway. In order to identify novel molecular signaling pathways critical for regeneration, we developed a rapid throughput in vivo regeneration assay. We screened a 2000 member structurally diverse small molecule library, followed by assessment of regenerative progression at three days post amputation. A cluster of glucocorticoids was identified among the "positive hits". Further studies with Beclomethasone, one of the "positive hits" revealed that transient activation of glucocorticoid receptor (GR) during early stages of regeneration was required to block regeneration. Glucocorticoids inhibited wound healing and blastema formation and affected cell proliferation at multiple regenerative phases. Since glucocorticoids are known anti-inflammatory agents, we analyzed the role of neutrophils and macrophages during regeneration by antisense repression of the Pu.1 expression, a factor required for myeloid cell development. We found that the acute inflammatory response by neutrophils and macrophages is not required for tissue regeneration. To identify the molecular targets of the activated glucocorticoid receptor, microarray analysis was performed using RNA isolated from the regenerates of control and glucocorticoid exposed zebrafish. We identified 673 transcripts that were differentially regulated. The level of expression and spatial expression pattern of select genes were completed by qPCR and by in situ hybridization, respectively. Altogether, these studies demonstrate the power of chemical genetics to identify chemical probes and their targets which will provide a path towards defining conserved regenerative pathways.

2197 INHIBITOR OF DNA BINDING-1 IS REQUIRED FOR NORMAL ZEBRAFISH CAUDAL FIN REGENERATION.

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The Id proteins (Inhibitor of DNA binding) are a class of helix-loop-helix (HLH) proteins that lack a DNA binding domain and function by forming inactive heterodimers with other HLH proteins. The Id proteins promote G1/S phase transition of the cell cycle and are important regulators of lineage commitment and terminal differentiation. Previous findings from our lab show that retinoic acid signaling regulates the expression of Id proteins. Dietary derived all-trans retinoic acid (atRA) is the main signaling retinoid in the body and is vital for biological functions such as embryogenesis, growth and differentiation, as well as vision and reproduction. The effects of retinoic acid during regeneration have been well studied in a variety of models including chick, frog, and zebrafish. In zebrafish fin regeneration, retinoic acid has been shown to be both teratogenic and morphogenic, causing either narrowing of the fins and bony ray fusion or increased number of ray segments.To date, very little data exists describing the role of Ids during the regeneration process. We hypothesize that zebrafish inhibitor of DNA binding-1 (Id1) is critical in caudal fin regeneration and is a target of retinoid signaling. Id1 expression is induced during regeneration in the zebrafish caudal fin and this increased expression is associated with the blastemal tissue. We also demonstrate that exposure to atRA during regeneration results in decreased Id1 expression at 2 and 4 days post amputation. In addition to decreased expression, the expression pattern of Id1 is diffused across the poorly organized regenerated tissue and highlights the lack of distinct blastemae normally associated with individual fin rays. Taken together, this suggests that Id1 may be an important intermediate in retinoic acid signaling and play an essential role in the effect of atRA on caudal fin regeneration.

2198 ROLES OF ARYL HYDROCARBON RECEPTORS (AHR) AND AHR REPRESSORS IN DEVELOPMENT AND DEVELOPMENTAL TOXICITY OF TCDD.

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The aryl hydrocarbon receptor (AHR), a member of the basic helix-loop-helix-Per-ARNT-Sim (bHLH-PAS) protein family, is a ligand-activated transcription factor that mediates transcriptional and toxic responses to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The AHR repressor (AHRR) is a closely related bHLH-PAS family member that forms a negative feedback loop to repress AHR signaling. Due to a teleost-specific genome duplication, zebrafish (Danio rerio) have three AHR (AHR1a, AHR1b, AHR2) and two AHRR (AHRRa, AHRRb) genes. To characterize novel roles of AHR and AHRR in regulating zebrafish development, morpholino oligonucleotides (MO) targeting AHR1b, AHR2, AHRRa and AHRRb were employed in zebrafish embryos. Embryos were micro-injected with MO at the 2-4 cell stage and exposed to 0.1 % DMSO or 2 nM TCDD at 6 hours post fertilization (hpf) for one hour. The prevalence of developmental phenotypes was documented at 48 and 96 hpf. Consistent with previous studies, the MO targeting AHR2 protected larvae from TCDD-induced edema and other developmental defects. In contrast, a MO targeting AHR1b did not provide any protective effects against TCDD-induced developmental phenotypes. Developmental phenotypes resembling those caused by TCDD (pericardial edema and heart elongation) were observed in DMSO-treated embryos microinjected with a MO targeting AHRRa in 62% and 67% of the embryos at 48 and 96 hpf, respectively. Furthermore, AHRRa knockdown significantly enhanced TCDD-induced pericardial edema at 48 hpf. In contrast, no developmental phenotypes were observed in DMSO-treated AHRRb knockdowns at either time point and TCDD-induced pericardial edema was not enhanced. These data suggest an AHR2-specific pathway for TCDD-induced developmental defects and that AHRR may modulate constitutive AHR signaling during normal development. (R01ES006272)

2199 SELDI PROTEINCHIP-BASED LIVER BIOMARKERS IN FUNGICIDE EXPOSED ZEBRAFISH.

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Sponsor: T. Reddy.

The research presented here is part of a three-phased small fish computational toxicology project using a combination of 1) whole organism endpoints, 2) genomic, proteomic, and metabolomic approaches, and 3) computational modeling to (a) identify new molecular biomarkers of exposure to endocrine disrupting compounds (EDCs) representing several modes of action (MOA) and (b) link these biomarkers to effects that are relevant for both diagnostic and predictive risk assessments. The effects of prochloraz (PCZ) on reproductively mature male and female zebrafish were examined following 48 h continuous exposure in a flow-through system to

control (water only), low (100 μg/l), and high (500 μg/l) doses. PCZ is an imidazole fungicide used to protect food crops by weakening fungal cell membranes through CYP51 inhibition. PCZ is known to have a mixed MOA in vertebrates, functioning as an aromatase (CYP19) inhibitor as well as an androgen receptor antagonist. Zebrafish liver lysates were profiled on three SELDI ProteinChip array types (weak cation exchange, strong anion exchange, and metal affinity), focusing on the 1.5 kDa to 25 kDa mass range. Over 300 individual m/z peak clusters were analyzed across the six sample groups (n=8 each). Gender related protein expression differences ($p<0.05$) were observed, with a majority of these markers exhibiting higher expression in males. Both up- and down-regulated markers were detected upon PCZ exposure. PCZ response in the females was observed primarily as up-regulation of candidate markers; in contrast, considerably more changes, primarily down-regulation of candidate markers, were observed in males. These differential expression profiles will be used to help identify new molecular biomarkers of exposure and integration of these data with whole organism endpoints will provide more definitive description of the mixed MOA of PCZ.
Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy.

2200 VASCULAR DILATION AND ANEURYSM DETECTED BY NONINVASIVE HIGH-FREQUENCY ULTRASONOGRAPHY IN APOE DEFICIENT MICE INJECTED WITH ANGIOTENSIN II.

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INTRODUCTION:Subcutaneous(SC) infusion of angiotensin II(AngII) in apolipoprotein E deficient(apoE-/-) mice promotes vascular remodeling, including vascular dilation and aneurysm. Ultrasonography(US) is commonly employed in a clinical to assess vascular anatomical change and hemodynamic dysfunction. The aim of this study was to assess the ability of a high-frequency US system, Vevo 770(VisualSonics.) to visualize the vascular injury in mice.
METHODS:Twenty-five male apoE-/- mice at 8 weeks of age were used. Over 4 weeks, apo E-/- mice fed on a high fat diet received AngII(1.44 mg/kg/day, SC) or vehicle using osmotic pumps. Blood pressure(BP) was monitored continually by a telemetry system. US examination was performed on the abdominal aorta(AA) and renal artery(RA). Assessment of vascular dilation and aneurysm was carried out with B-mode US. Assessment of blood flow velocity for evaluation of hemodynamic function was also performed using US pulsed-Doppler(PD) mode. During the PD assessment, peak systolic and end-diastolic velocities(Vs, Vd) and resistive index(RI) were obtained. Pathological examination was performed at the end of the experiment.
RESULTS:AngII-induced vascular dilation was seen in the AA and RA by 164.39 % and 122.6 % respectively. From the first week of treatment, thoracic aortic aneurysm(TAA) and abdominal aortic aneurysm(AAA) showing severe vascular dilation of >200% were observed in one and three mice out of fifteen mice, respectively. Thereafter, both aneurysms were diagnosed pathologically as dissecting aneurysms. Hemodynamic assessment showed increased RI and reduced Vs and Vd in the AA and RA.
CONCLUSION:We could detect vascular remodeling, such as vascular dilation, hemodynamic dysfunction and aneurysms in AngII-treated apoE-/- mice with the high frequency US system. It is concluded that the high frequency US system is a valid method of vascular dysfunction assessment in noninvasive longitudinal rodent studies.

2201 INFLAMMATORY PROTEIN AND GENE EXPRESSION IN MONOCYTES AND MACROPHAGES EXPOSED TO CHOLESTEROL SECOALDEHYDE: IMPLICATIONS TO ATHEROSCLEROTIC PLAQUE DEVELOPMENT.

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Cholesterol secoaldehyde (ChSeco), an oxysterol known to be formed *in vitro* in reactions of cholesterol with ozone and not during autoxidation, has been reported to be present in human atherosclerotic plaque up to 60 μM! While the source as well physiological significance are largely unknown, there has been a growing interest in this "ozone-specific" oxysterol as a promoter of atherosclerotic plaque in particular and a mediator of cytotoxicity in general. For instance, ChSeco has been shown to trigger foam cell formation, causes protein misfolding, and is cytotoxic to numerous mammalian cell types including cultured macrophages, bronchial epithelial cells, neuronal cells, vascular endothelial cells, and cardiomyocytes. As part of understanding the role of ChSeco in atherosclerosis, in the present study, we examined the inflammatory protein (cytokines) and their gene expressions in human monocytes (THP-1) and mouse macrophages (J744). It was found that, in THP-1 cells exposed to ChSeco at concentrations close to IC_{50} for 24 h, there was a marked increase in the expression of IL-8 (156 ± 4 pg/mL; M ± SD, n = 3) but not TNF-α. Real time PCR (SuperArray) analysis revealed over expression of 10-15 pro-inflammatory genes (e.g., *Il1r*, 8.5-fold; *Ccl20*, 7.9-fold; and *Casp1*, 3.1- fold) and down regulation of three anti-inflammatory genes (viz., *Il1β*, 10.3 fold; *Il10*, 3.1-fold; and *Lym-B*, 3.2-fold) in J744 cells exposed ChSeco. It is suggested IL-8 secreted by monocytes and, possibly, macrophages play a significant role in the atherosclerotic plaque development through recruiting T lymphocytes to the lesion. [Funding support from NIH (grant number P20 RR 16456) and NSF (grant number HRD 0450375) to RMU is acknowledged; corresponding author, e-mail: rao_uppu@subr.edu.]

2202 EFFECTS OF PRENATAL CIGARETTE SMOKE EXPOSURE ON LIPID PARAMETERS ASSOCIATED WITH OFFSPRING CARDIAC RISK: A STUDY IN MICE.

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Interest in the subsequent effects of early toxic insult on disease vulnerability of the adult offspring is increasing. This study examined the effects of prenatal exposure to mainstream cigarette smoke (MCS) on cardiovascular-associated lipid parameters in adult offspring fed either normal chow or a high fat diet (HFD) for 2 wk. Plasma levels of cholesterol (CHO), triglycerides (TRI), phospholipids (PPL), high (HDL) and low density lipoprotein (LDL), albumin (A), and total protein (TP) were determined in 13-14-wk-old male and female offspring exposed gestationally (4 h/d, 5 d/wk) to air or MCS at a concentration equivalent to smoking <1 pack of cigarettes/d. Female (but, not age-matched male) offspring exposed prenatally to MCS and fed a normal diet demonstrated increased HDL (24%), LDL (24%), and TP (6%) levels compared to sex-matched air-exposed mice. Interestingly, female offspring of smoking mothers also weighed 10% more than control pups. Alternatively, when prenatally MCS-exposed offspring consumed a HFD for 2 wk prior to sacrifice, a number of lipid parameters (i.e., CHO, HDL, PPL, and A) were significantly increased (by 10-40%) in the male (but, not female) offspring compared to their sex-/diet-matched air-exposed counterparts. Thus, while adult female mice on a normal diet appeared most sensitive to the lipid-altering effects of prenatal MCS exposure, male offspring proved even more vulnerable when challenged subsequently with a second cardiac disease risk factor (i.e., HFD). Given that changes in lipid homeostasis may indicate alterations in CHO metabolism which could promote vascular changes associated with atherosclerosis, these studies suggest that offspring (particularly, male) exposed prenatally to MCS and stressed subsequently as adults by a HFD may be at greater risk for cardiovascular disease than those from non-smoking mothers. Supported by Philip Morris and NIEHS

2203 MECHANISMS OF INHIBITION OF VASCULAR CALCIFICATION BY PHOSPHONOFORMIC ACID (FOSCARNET) IN RAT VASCULAR SMOOTH MUSCLE CELLS.

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Medial vascular calcification (VC) is caused by the trans-differentiation of vascular smooth muscle cells (VSMC) into osteoblast-like cells as a response to hyperphosphatemia. The role and response of inorganic phosphate (Pi) transporters to high Pi serum levels has been previously determined by inhibition with phosphonoformic acid (PFA). We have observed that 1 mM PFA completely prevents calcification of VSMC induced with 2 mM phosphate. However, PFA is a very poor inhibitor of Pi transport in VSMC from rat aorta (Ki of 2.6 mM, while the Km for Pi is 0.1 mM) and of Pit1 and Pit2 Pi transporters when expressed in Xenopus laevis oocytes. In order to know the mechanism of VC by PFA, cytotoxicity of VSMC was then analyzed. Phase-contrast microscopy revealed that cells treated with 10 mM PFA for several days were larger and less confluent, nevertheless no signs of cell death could be determined with 1 mM PFA using either a cytosolic LDH assay, double staining with acridine orange / ethidium bromide, or a fluorescent TUNEL assay. A likely inhibition of adenylyl-cyclase and guanylyl-cyclase by PFA as reported was also analyzed to explain prevention of calcification. This possibility was also discarded because VC prevention could not be reproduced with different pharmacological inhibitors of cyclases, nor it was blunted with non-metabolizable cAMP and cGMP analogs. Finally we also checked a direct effect of PFA (as a phosphonate) in the extracellular deposit of hydroxyapatite. VSMC were induced to calcify with 2 mM Pi

for 1-5 days, while lysing them with formalin or distilled water at days 1-5, and maintaining the calcification induction for the remaining time, ie. up to 5 days. Lysed cells calcified as much as live cells, and this was still successfully prevented by PFA. In conclusion, PFA prevents vascular calcification by acting directly on the CaxPi hydroxyapatite formation.

2204 ARYL HYDROCARBON RECEPTOR-DEPENDENT ALTERATIONS IN HEPATIC AND VASCULAR GENE EXPRESSION IN ADULT ZEBRAFISH.

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Developmental exposure to aryl hydrocarbon receptor (AhR) agonists in fish causes severe defects in the cardiovascular system. However, the effects of acute AhR agonist exposure on the adult fish cardiovascular system are unknown. We hypothesized that AhR-mediated changes in adult vascular tissue gene expression would differ from that of hepatic tissue. Therefore, zebrafish (*Danio rerio*) were intraperitoneally injected with the AhR agonists benzo(a)pyrene (BaP; 0.1 mg/kg) or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; 20 ng/kg) separately and in combination with the AhR antagonist resveratrol (1.0 mg/kg). Hepatic and vascular cytochrome P450 enzyme (subtypes 1A, 1B1, 1C1, and 1C2) mRNA expression was quantified using real-time reverse transcriptase PCR. The current study found that TCDD exposure significantly ($p \leq 0.05$ in Tukey's posteriori after 2-way ANOVA; n=5-6/group) increased CYP1A, CYP1B1, and CYP1C1 mRNA expression in hepatic tissue (fold increases of 90±21, 2±0, 32±6). TCDD also increased CYP1A, CYP1C1, and CYP1C2 mRNA expression in vascular tissue (fold increases of 71±14, 27±6, 5±1). Importantly, while BaP exposure elicited no significant alterations in hepatic mRNA expression, BaP exposure significantly increased CYP1B1 expression in vascular tissue (fold increase of 2±1). Resveratrol was without effect in all treatments in both hepatic and vascular tissue. Thus either the resveratrol dose was insufficient to protect against BaP and TCDD effects or these toxicants exert effects independently of AhR. The vascular-specific changes in gene expression will be linked to future studies examining alterations in cardiovascular function produced by acute AhR exposure in adult fish.

2205 VASCULAR SMOOTH MUSCLE DYSFUNCTION INDUCED BY GINSENOSIDE RG3, A BIOACTIVE COMPONENT OF GINSENG.

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Complementary and alternative medicines (CAM) are gaining more and more popularity among cardiovascular disease stricken patients, raising concerns for their abuse and toxicities. We investigated the effect of ginsenoside Rg3 (Rg3), the active component of the famous herbal medicine, ginseng, on the contractile function of vascular smooth muscle and elucidated its underlying mechanism to find any relevance to vascular dysfunction caused by CAM. In organ bath system using isolated rat thoracic aorta, pretreatment of Rg3 attenuated vessel contraction induced by contractile agonists, phenylephrine (PE), serotonin and endothelin-1. Inhibitory effect of Rg3 was due to the disruption of PE-induced cytosolic Ca^{2+} elevation in smooth muscle cells. In addition, suppression of Ca^{2+} mobilization by Rg3 was confirmed to be from blocking of L-type Ca^{2+} channel current using the patch clamp assay in Xenopus Oocytes. Conspicuously, these inhibitory effects on vasoconstriction and Ca^{2+} influx were not reversed even after the removal of Rg3, a striking difference from anti-hypertensive drug, nifedipine, suggesting that Rg3 impaired the function of vascular smooth muscle permanently. In *in vivo* rat model, Rg3 attenuated PE-induced blood pressure increase indeed, supporting the relevance of these *in vitro* results. In summary, Rg3 was shown to irreversibly suppress agonist-induced vasoconstriction by blocking of Ca^{2+} influx through L-type Ca^{2+} channel. The irreversible effects of Rg3 could be distinguished from the reversible effects of conventional anti-hypertensive drug, nifedipine, which could be referred as smooth muscle dysfunction. This work will provide new insights for the understanding of potential vascular dysfunction by CAM.

2206 SUBCHRONIC EXPOSURE OF ADULT MICE TO 2, 3, 7, 8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD) INCREASES BLOOD PRESSURE AND ALTERS VASCULAR REACTIVITY.

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Hypertension is a prevalent disorder in industrialized regions. Human epidemiology studies have correlated TCDD exposure with cardiovascular disease, including hypertension. Thus, we tested the hypothesis that subchronic TCDD exposure increases arterial blood pressure, a risk factor for cardiovascular disease-related mortality. A radiotelemeter surgically implanted in the left carotid artery of 3.5 mo old male C57Bl/6N mice was used to measure blood pressure. Mice were dosed with vehicle control (corn oil) or 150 ng/kg TCDD by oral gavage three times per wk for 60 d, approaching a steady state body burden of 1 μg/kg. Both daytime and nighttime mean arterial pressure of TCDD-exposed mice significantly increased within 7 d, followed by normalization and subsequent increases, peaking at 35 and 60 d. Left ventricular hypertrophy was present after 35 and 60 d of treatment. Heme oxygenase-1 expression was significantly increased in kidney after 35 d, suggestive of oxidative stress. Vascular reactivity to acetylcholine revealed a decreased vasodilatory response in aortas of TCDD-exposed mice, which was restored in the presence of tempol, an SOD mimetic. These results suggest that subchronic TCDD exposure may be a risk factor for hypertension and that oxidative stress may be a key part of the underlying mechanism. Supported by NIH ES012335 and HL078914 to MKW; P30ES12072 to UNM; AFPE Pre-Doctoral Fellowship to PGK.

2207 HYPERTENSIVE HEART DISEASE IN OLDER ARYL HYDROCARBON RECEPTOR NULL MICE PROGRESSES TO SYMPTOMS OF HEART FAILURE.

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Aryl hydrocarbon receptor (AHR) null mice exhibit endothelin (ET)-1-dependent hypertension, cardiac hypertrophy, and cardiac and renal fibrosis at 5 mos. We tested the hypothesis that disease in AHR null mice would progress towards heart and renal failure with age. Blood pressure was determined in one-year-old male AHR null and wildtype mice by radiotelemetry and cardiac function was assessed by echocardiography. Blood chemistries related to renal function were determined; heart, lung, and kidney weights were measured; and vascular responses to ET-1 were assessed in aortic rings. Mean arterial pressure (MAP) was lower in AHR null mice compared to wildtype. Although fractional shortening was normal, left ventricular end diastolic diameter was increased as was left ventricular posterior wall thickness in diastole and systole. Heart and kidney weights were increased in AHR null mice, while lung weight and all blood chemistries (blood urea nitrogen, creatinine, sodium, and chloride) were normal. Finally, aortic rings from AHR null and wildtype mice exhibited equivalent dose-related contraction to ET-1. These results suggest that although older AHR null mice exhibit significant renal hypertrophy, renal function appears normal. In contrast, older AHR null mice develop symptoms consistent with the onset of heart failure, including hypotension, cardiac hypertrophy (worse than at 5 mo), and left ventricular diastolic dilation (absent at 5 mo). Supported by HL078914 to MKW.

2208 NON-INVASIVE BLOOD PRESSURE MONITORING IN THE CYNOMOLGUS MONKEY (MACACA FASCICULARIS): HIGH DEFINITION OSCILLOMETRY *VERSUS* IMPLANTED TELEMETRIC DEVICES.

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Currently, approaches for accurate blood pressure determination rely predominantly on invasive techniques. This holds particularly true for safety pharmacology studies. The present work evaluates the High Definition Oscillometry (HDO) technique as a potential non-invasive approach for accurate blood pressure recordings. HDO enables visible, real time control of each measurement on a screen by the use of blood pressure amplitude scans with up to 16.000 Hz within 10 – 15 sec. A 32-bit processor allows real-time analysis and real-time electronic valve programming in the 5 – 300 mmHg pressure range for individual pressure calculations within microseconds. In a previous study, even heart rates higher than 400 beats per minute imposed no limitations for HDO measurements (1). Six male cynomolgus monkeys, chronically implanted with a Koenigsberg T27 cardiovascular transmitter (DISS, Dexter, USA) were used for simultaneous telemetric and HDO measurements. For each individual the systolic and diastolic blood pressure, mean arterial pressure and the pulses/min were determined in 1 minute intervals. In order to clarify the ability of each system to detect cardiovascular changes, animals were anesthetized after 4 to 10 minutes of interval measurements by Ketamine-Xylazine (Ketamine 10 mg/kg, Xylazine 0.25 – 0.3 mg/kg). Both systems revealed an approximately twofold decrease of heart rate under anesthesia. Twenty five minutes after dosing a comparable and significant reduction of systolic blood pressure compared to predose values was detected by both approaches (HDO: mean (± SD) delta mmHg: 51.4 ± 4.4 ; telemetry: mean delta mmHg: 60.1 ± 4.0). Our results demonstrate the feasibility of non-invasive blood pressure measurements by the HDO technique and - when compared to telemetry - the ability and accuracy to detect drug related cardiovascular changes.

1. Schmelting B, Egner B, Korte SH, Weinbauer GF 2007. J Am Ass Lab Animal Sci Vol 46(4), 144

2209 EFFECTS OF NO MODULATORS ON CARDIOVASCULAR RISK FACTORS IN MILD HYPERHOMOCYSTEINEMIC RAT MODEL.

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Objectives: Hyperhomocysteinemia(Hhcy) is considered to be an independent risk factor in atherosclerosis. In the present paper we observed the effect of NO modulators on cardiovascular risk factors in mild hyperhomocysteinemic rats.
Design and methods: A rat model of mild Hhcy was established by administering different doses of methionine(0.1g/kgbwt, 0.25g/kgbwt, 0.5g/kgbwt,1g/kgbwt orally) for 8 weeks. The appropriate dose of methionine was administered to rats with concomitant treatment with Sodium nitroprusside and Nω nitro-L-arginine during the induction of Hhcy. Lipid profile, total antioxidant capacity and the level of homocysteine and NOx(Nitrates and nitrites) was examined in serum at 0 and 4 weeks. Activity of HMG-CoA reductase, the mRNA level of Caveolin, P2 receptors and Cardiovascular risk factors were also analyzed.
Results: Stimulated lipid profile of rats by the treatment of methionine(1g/kg bwt) reduced significantly by the treatment of SNP with methionine. LNNA increased the level of cholesterol in aorta($p<0.05$vs group II). SNP significantly suppressed the activity of HMG-CoA reductase. The mRNA levels of caveolin($p<0.05$), P2X($p<0.05$) and P2Y($p<0.05$) showed a significant decrease in rats administered with SNP. LNNA showed significant induction in the expression of caveolin ($p<0.01$) and P2Y($p<0.01$) expression. The level of P2X showed no remarkable change in animals treated with LNNA and methionine both.
Conclusions: This data concludes that NO modulators modulate the effect of hyperhomocystinemia on the other cardiovascular risk factors and confirms the finding that nitric oxide play important role in homocysteine induced cardiovascular diseases.

2210 15-DEOXY-Δ12, 14 PROSTAGLANDIN J2 INDUCES PLATELET FORMATION IN A HUMAN MEGAKARYOBLASTIC CELL LINE.

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Bleeding due to thrombocytopenia is a critical problem that occurs during certain diseases and after myeloablative therapy for cancer. Loss of platelets can also occur after radiotherapy prior to bone marrow transplant and following a radiation terror attack. Treatments for platelet loss include platelet transfusions and administration of recombinant human Interleukin (IL) -11. However, these therapies are sometimes insufficient for disease management because of problems with infection and drug administration. Thus, the development of safe, small molecules to enhance platelet production would be advantageous for the treatment of thrombocytopenia. Herein, we report that an important lipid mediator called 15-deoxy-Δ12,14 prostaglandin J2 (15d-PGJ2) causes the Meg-01 cells, a human megakaryoblastic cell line, not only to mature, but also to produce more platelets. Although redox shift can regulate the development of the cells, including proliferation, differentiation, and survival, the role of reactive oxygen species in platelet production remains unclear. Many disorders associated with oxidative stress are also associated with elevated platelet number such as type-2 diabetes mellitus and atherosclerosis. We demonstrate that 15d-PGJ2 induces reactive oxygen species (ROS) and superoxide accumulation and that incubation with glutathione reduced ethyl ester, an antioxidant, attenuates 15d-PGJ2-induced platelet production. Collectively, these data support the concept that megakarocyte redox status plays an important role in platelet generation. This information will be important to understanding the platelet changes that occur during chronic inflammatory diseases such as type-2 diabetes and cardiovascular disease. In addition, discovering alternative treatments for increasing platelet number will reduce the risk of life-threatening hemorrhage in thrombocytopenic patients.

2211 AN INVESTIGATION OF PARAOXONASE-1 ($PON1_{192}$) ACTIVITIES IN THE SERUM OF SOUTHERNERS AS RELATED TO GENDER AND RACE.

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Paraoxonase-1 (PON1) has an anti-oxidative function in preventing the formation of oxidized lipoproteins (LDL and HDL) and hydrolyzing the active metabolites of some organophosphate insecticides (e.g., paraoxon and diazoxon) and other non-physiological substrates. $PON1_{Q192R}$ affects PON1 hydrolytic activity and its protective role against oxidative stress, thereby influencing susceptibility to cardiovascular disease among individuals. Human studies of $PON1_{192}$ activities have not been conducted on Southern or many African American populations, so neither the activity levels nor the proportion of the genotypic forms (QQ, QR, and RR) are known among these individuals. The objectives of this study are to determine the effect of race and gender on paraoxonase (POase) and diazoxonase (DZOase) activities in Caucasian and African American Southerners. Serum samples from 200 individuals (equally distributed race and gender classes, serum obtained from individuals in Tennessee and Alabama, ages 25-65) were assayed spectrophotometrically for paraoxon and diazoxon hydrolysis. Data indicate a positive correspondence between $PON1_{192}$ genotypes and race and PON1 activity and race. Results show that Caucasian Southerners have a mean DZO/POase ratio twice that of African American Southerners. When comparing frequency distribution of $PON1_{192}$ genotypes, more Caucasians (~60%) possess the genotype more protective of cardiovascular health (QQ) and more African Americans (~85%) possess the genotypes less protective of cardiovascular health (QR and RR). African American Southerners (both genders) have higher paraoxonase activities and lower diazoxonase activities than Caucasian Southerners (both genders). Data do not indicate an influence of gender or age on PON1 activity. These results should be useful in explaining the increased risk of cardiovascular disease in African Americans compared to Caucasians. (Supported by NIH R21 ES015107).

2212 CHARACTERIZATION OF ENDOTHELIAL DYSFUNCTION INDUCED BY NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR ANALOGUES AND THEIR PHOSPHORYLATED METABOLITES.

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Nucleoside reverse transcriptase inhibitors (NRTI) are considered the backbone for HIV combination drug therapy. Our prior studies in rodents indicated that NRTI, alone or in combination with other antiretrovirals, can induce endothelial dysfunction (ED) after chronic administration. While ED is the first step and a sensitive marker for atherosclerosis and other cardiovascular diseases, mechanistic studies in our laboratory demonstrated that the NRTI Zidovidine (AZT) induced endothelial dysfunction dependent upon an increase in mitochondrial ROS production. NRTI include thymidine, cytidine and adenosine nucleoside analogues. Studies are required to determine the observed effects are specific to AZT or whether the mitochondrial mechanism is shared by other nucleoside analogues. Further, we hypothesized that the phosphorylated metabolites of NRTI can induce more severe endothelial toxicity than the parent compounds. To test our hypothesis, we used in vitro cultures of human umbilical vein endothelial cells treated with various nucleoside analogues or their phosphorylated metabolites. Our results indicated that NRTI including thymidine analogues like AZT and stavudine, cytidine analogues such as lamivudine, and adenosine analogues like didanosine can significantly and time-dependently increase ROS production, inactivate aconitase, and increase the lactate/pyruvate ratio, suggesting mitochondrial dysfunction. Overall, the thymidine analogues were not more toxic than other subclasses of antiretrovirals, and the level of ROS production was not dependent upon the subclass of NRTI. Further, by comparing the phosphorylated metabolites to the parent compound, we determined that AZT-triphosphate induced a much greater increase in ROS production than AZT itself. Ongoing studies are aimed at determining the effect of these various NRTI nucleoside analogues on discrete mitochondrial targets to assess whether there is a common mechanism for endothelial toxicity shared by this class of drugs.

2213 ANTIRETROVIRALS INDUCE ENDOTHELIAL DYSFUNCTION *VIA* AN OXIDANT-DEPENDENT PATHWAY AND PROMOTE NEOINTIMAL HYPERPLASIA.

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HIV antiretrovirals, such as nucleoside reverse transcriptase inhibitors (NRTI) and protease inhibitors (PI), have now been shown associated with premature atherosclerosis, even in young patients without other risk factors. An early endothelial dysfunction and a subsequent vascular smooth muscle cell proliferation are two important events in atherogenesis. We hypothesized that antiretrovirals exacerbate atherogenesis by inducing endothelial dysfunction and promoting neointimal hyperplasia. C57BL/6 mice were dosed with two antiretrovirals, the NRTI AZT (100 mg/kg), the PI indinavir (100 mg/kg), or AZT plus indinavir, for 5 days. Endothelial dysfunction was assessed in aortic rings as a reduction in acetylcholine-dependent vessel relaxation. AZT or AZT plus indinavir treatment significantly compromised endothelial function, but this effect was attenuated by co-administration of MnTMPyP, a cell permeable antioxidant. This suggests that AZT- or AZT plus indinavir-mediated endothelial dysfunction was due to an increased oxidant production. To determine whether these two antiretrovirals promote vascular

neointimal hyperplasia, the proliferative response that generally follows endothelial dysfunction, C57BL/6 mice were treated with antiretrovirals plus a Western diet for 4 weeks. Two weeks following the initiation of treatment, a carotid artery injury procedure was performed to denude the endothelium, and the mice were again administered a Western diet plus antiretrovirals. At 14 days after injury, a significant neointimal layer was formed in the carotid artery. AZT, indinavir, and AZT plus indinavir treatment markedly promoted this neointimal hyperplasia. BrdU fluorescence staining confirmed that the thickening of the neointimal layer was due to increased proliferation of vascular smooth muscle cells. Results of this study thus suggest that the antiretrovirals AZT and indinavir initiate and/or promote atherogenesis by inducing oxidant injury in the vascular endothelium and consequently, promote neointimal hyperplasia.

2214 MANUFACTURED NANOPARTICLES INDUCE ENDOTHELIAL CELL INFLAMMATION.

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Nanomaterials are promoted as a promising technology in highly diverse applications, but concerns about risks of these materials have motivated research on the toxicology of manufactured nanoparticles. Our ongoing research utilizes in vitro cultures of human-derived cells, treated with suspensions of low-solubility metal oxide materials, to study uptake, cell signaling, and gene regulation. Environmental exposure to these particles is primarily through inhalation. However, ingestion or absorption through the skin also occurs. The effects on the cardiovascular system occur because inhaled nanoparticles may evade phagocytosis, cross cell membranes, and redistribute to other sites of the body, causing systemic health effects. Silicon dioxide (SiO2) particles in the range of 20-100 nm were used in our studies because they have many commercial and medical applications and can be readily modified with surface functional groups. Our hypothesis is that SiO2 nanoparticles cause acute phase responses in vascular endothelial cells, leading to altered production of nitric oxide (NO). Our goal is to elucidate the mechanisms by which nanoparticles induce the production of inflammatory mediators and alter vasodilatory mediators in vascular endothelial cells. The results presented here describe the uptake, inflammatory response and alterations in NO and NO-producing enzymes in vascular endothelial cells after exposure to nanosilica. The 100 nm nanosilica particles were incorporated into cells, and also attached to the surface of vascular endothelial cells. Nanosilica caused a robust proinflammatory response in human endothelial cells at low concentrations and cell death at higher concentrations. Particle-treated cells also showed a decrease in endothelial nitric oxide synthase (eNOS) and an increase in C-reactive protein (CRP) mRNA which may be the cause of the altered vasodilatation in patients exposed to particulate matter. Our results suggest novel mechanisms by which to explain nanosilica effects on the cardiovascular system. This work was supported by EPA STAR RD8317230 and NIEHS K25 ES 011281.

2215 ENDOTHELIAL CELL CAVEOLAE ARE INVOLVED IN ACTIVATION OF ARYL HYDROCARBON RECEPTOR PATHWAY BY COPLANAR POLYCHLORINATED BIPHENYLS.

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Polychlorinated biphenyls (PCBs) are ubiquitous and persistent environmental pollutants. Epidemiological and experimental data have suggested that PCBs play a significant role in development of cardiovascular pathologies, such as atherosclerosis. Caveolae are membrane microdomains that mediate endocytosis and regulate multiple signaling pathways involved in atherosclerosis, in particular in endothelial cells. We hypothesized that caveolae play an important role in endothelial activation by coplanar PCBs. Primary endothelial cells exposed to PCB77 had increased expression of caveolin-1, a major structural component of caveolae. This caveolin-1 up-regulation resulted in enrichment of caveolin-1 in the caveolae fraction collected by sucrose gradient isolation, as well as increased membrane caveolae formation demonstrated by transmission electron microscopy. Next we evaluated whether functional caveolae are required for PCB77 toxicity mediated by activation of the aryl hydrocarbon receptor (AhR) and up-regulation of downstream genes such as cytochrome P450 1A1 (CYP1A1). AhR was found to co-immunoprecipitate with caveolin-1, suggesting that this scaffolding protein plays a role in regulation of activation of AhR and associated genes. Compared to control cells, PCB77 failed to induce CYP1A1 expression in endothelial cells after caveolin-1 was silenced by siRNA. Similarly, in liver of caveolin-1 null mice a significant decrease in PCB77-induced CYP1A1 expression was observed compared to control mice. In conclusion, these data demonstrate that coplanar PCB77 induces caveolae formation and that the presence of functional caveolae is required for activation of the AhR pathway in endothelial cells. (Supported by grants from NIEHS, NIH (P42ES07380) and UK AES)

2216 ENVIRONMENTAL PCBs INCREASE BLOOD VESSEL FORMATION BY OXIDANT SIGNALING.

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Estrogen is a known mitogen of endothelial cells (ECs). Since PCBs are reported to mimic estrogens, it is plausible for PCBs to mediate a similar vasculoproliferative response. There is now increasing evidence that exposure to PCBs can contribute to the development of atherosclerosis. How PCBs may support or promote occlusive vascular lesions is not clear. Since the overproduction of reactive oxygen species (ROS) is a major hallmark of cardiovascular disease, we examined whether PCB exposure of ECs increase the formation of ROS and the role of ROS in blood vessel formation. Methods: The effect of PCBs on the production of ROS and the role of PCB-induced ROS on vessel formation was studied in human umbilical vein ECs. ROS were measured by monitoring the oxidation of 2'7'-dichlorofluorescin. EC vessel formation was determined in Matrigel, co-culture, and cell proliferation was measured by BrdU assay. Results: PCB 153 was tested at equivalent concentrations reported to be found in human serum. PCB exposure triggered a rapid increase in intracellular oxidants in ECs. PCB treated ECs showed a significant induction of capillary tube formation in both Matrigel and when co-cultured with fibroblasts. PCB exposed ECs showed a significant increase in DNA synthesis at 18 h when compared to control. Overexpression of the antioxidant enzyme catalase and co-treatment with the chemical antioxidant ebselen showed a significant inhibition of PCB induced capillary tubes. Conclusion: We have shown that PCB exposure stimulates the rapid production of intracellular ROS and they are involved in signaling blood vessel formation. It appears that the early PCB signaling does not require estrogen receptor genomic signaling because we can inhibit PCB-induced capillary tube formation by antioxidants. Findings of this study highlight the potential damaging consequences of PCBs to the vascular system. Since PCBs have been identified in human adipose tissue, blood, and milk; a risk for population exposure is possible and warrants further research to understand the role of PCBs in vasculoproliferative disorders.

2217 ARSENIC SIGNALS THROUGH A GαI-COUPLED PATHWAY TO INDUCE ANGIOGENIC GENES IN HUMAN MICROVASCULAR ENDOTHELIAL CELLS.

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Environmental arsenic (As(III)) exposure from drinking water is major global health problem that causes vascular remodeling, ischemic vascular disease, and hypertension in millions of people world-wide. The vascular endothelial cells are known primary targets of As(III). However, the mechanisms for initiating pathogenic endothelial cell signaling in response to low environmental levels of As(III) remain unresolved. Since many of the intermediate As(III)-stimulated signaling steps, such as Rac-1-GTPase and NADPH oxidase activation, occur within minutes of exposure, we hypothesized that As(III) initiates pathogenic phenotypic change through a signal amplification pathway. This hypothesis was tested by investigating signaling cascades mediating arsenic induction of angiogenic genes in human microvascular endothelial cells. RT-PCR arrays conducted with mRNA isolated from cells exposed to 1 μM As(III) for 4 or 24 h demonstrated that As(III) induced four and repressed three of the 84 angiogenic genes examined. The analysis was repeated with extracts from cells incubated for 18 h in the absence or presence of 50 μM pertussis toxin (Ptx), a selective inhibitor of Gαi-GTPase, prior to being exposed to As(III). The effects of As(III) on the 7 responsive genes were inhibited in the Ptx-treated cells indicating that these As(III) were mediated by Gαi-coupled signaling pathway. In separate RT-PCR assays, As(III) stimulation of heme oxygenase-1 mRNA levels was shown not to be inhibited by Ptx. This indicated that induction of the angiogenic genes was not related to stress activated signaling. Ptx inhibition was proximal to As(III)-stimulated Rac1 activity, which was required for induction of several of the As(III)-stimulated genes, including IGF-1 and IL-8. These data are the first to suggest that As(III) initiates pathogenic endothelial signaling for angiogenic and remodeling gene induction through a g-protein coupled signal amplification pathway. *Supported by NIEHS grant ES10638.*

2218 GENOTOXIC EFFECTS OF 1-NITROPYRENE IN HUMAN ENDOTHELIAL CELLS.

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Polycyclic aromatic hydrocarbons (PAHs) and nitrated PAHs are environmental pollutants found in diesel exhaust and ambient air. Exposure to air pollution is considered to contribute to an increased risk of cardiovascular disease. Cardiovascular

tissues have generally a limited ability to metabolize xenobiotics due to the low expression of drug metabolizing enzyme cytochrome P450 (CYP). Aryl hydrocarbon receptor (AhR) agonists such as PAHs, dioxins and coplanar PCBs can, however, induce CYP1A1 in endothelial cells suggesting that the ability of cardiovascular tissues to bioactivate PAHs and nitrated PAHs may be increased following exposure to CYP1A1-inducing environmental pollutants.
Nitropyrene (1-NP) is one of the most abundant nitrated PAHs in diesel exhaust particulate matter and it was selected as a model compound. The aim of the present study was to investigate the toxic effects of 1-NP on cultured human umbilical vein endothelial cells (HUVEC).
Our preliminary results revealed a dose-dependent genotoxic effect of 1-NP (1- 10 μM) in HUVEC cells as demonstrated by single cell electrophoresis (Comet assay). Higher levels of 1-NP resulted in decreased cell viability and increased formation of reactive oxygen species (ROS) as demonstrated by MTT assay and fluorescence-based enzyme assay
In conclusion, 1-NP induced DNA damage and oxidative stress in cultured HUVEC cells suggesting endothelial cells as a possible target site for nitro-PAHs. The effects of 1-NP in CYP1A1-induced HUVEC cells will also be studied.

2219 A NOVEL CXCR4 ANTAGONIST DERIVED FROM HUMAN SDF-1, SDF-1P2G, STIMULATES ANGIOGENESIS IN A MURINE MODEL OF ACUTE HINDLIMB ISCHEMIA.

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Emerging evidence demonstrates that SDF-1/CXCR4 axis plays a vital role in vascular injury recovery and angiogenesis through bone marrow stem cell mobilization and injury site-directed stem cell homing. However, multi-functions of wild type SDF-1 may limit its application for this purpose. Herein, we developed an antagonist of CXCR4, SDF-1P2G that was derived from human SDF-1 by replacing the second amino acid with glycine in the N-terminus.Treatment with SDF-1P2G (5 mg/kg) significantly stimulated angiogenesis in a murine model of acute hindlimb ischemia as early as 7 days after surgical operation, shown by expression of CD31+ cells/fiber ($p<0.001$,vs PBS). Comparison with AMD3100 (5mg/kg), another chemical antagonist of CXCR4, SDF-1P2G appears to stimulates a more rapid revascularization ($p<0.01$). Angiogenesis and stem cell migration related genes, including phosphor-Akt, VEGF, SDF-1 and CXCR4, were just co-localized with CD31 with high level expression in hindlimb gastrocnemius vs PBS treatment. Endothelial progenitor cells (CD31+ c-Kit+) in the peripheral blood of ischemia mice were analyzed by flow-cytometry at the day 1, 4 and 7 after surgical operation. Unexpected, there is no significant differences between SDF-1P2G and PBS treatments in CD31+ c-Kit+ cells percentage in blood ($p>0.05$), while AMD3100 induced an increase in CD31+ c-Kit+ cells percentage ($p<0.05$, vs PBS and SDF-1P2G). Collectively, these data suggest that the antagonist of CXCR4, SDF-1P2G, stimulates angiogenesis through distinct mechanisms from AMD3100 that is mediated by mobilization of the subset of CD31+ c-Kit+ cells from bone marrow. The exact mechanism needs to be further elucidated (The present study was supported in part by start-up grant from Chinese-American Research Institute for Diabetic Complications,and grant from American Diabetes Association,NSFC30600768,Y2006B003 and 2006A099).

2220 BENZO[A]PYRENE-INDUCED VASCULAR ENDOTHELIAL ADHESION MOLECULE EXPRESSION CAN BE DISRUPTED BY SELECTIVE FLAVONOID TREATMENT.

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Adhesion molecules, such as intracellular adhesion molecule-1 (ICAM-1), play a critical role in the initiation of vascular diseases such as atherosclerosis. Exposure to polycyclic aromatic hydrocarbons, such as benzo[a]pyrene (B[a]P) have been shown to correlate with the increased risk of cardiopulmonary diseases. Increased intake of dietary flavonoids may decrease the risk of developing these diseases. The goals of this research were to investigate the effects of B[a]P on endothelial cell adhesion and if flavonoids could protect against this endothelial pathology induced by B[a]P. Primary human umbilical vein endothelial cells (HUVECs) were pretreated overnight with vehicle or beta-naphthoflavone (β-NF) to induce aryl hydrocarbon receptor (AhR) regulated metabolizing enzymes. Cells were then treated with B[a]P for 24 hours. B[a]P increased ICAM-1 protein expression only when pre-treated with β-NF, suggesting B[a]P requires AhR to be converted into the active pro-inflammatory compound. Since ICAM-1 is necessary for the adhesion and migration of inflammatory leukocytes such as macrophages across the endothelium, macrophage adhesion was measured. Cells pretreated with β-NF and treated with B[a]P were able to induce adhesion of fluorescently labeled macrophages to the activated endothelium, a physiological representation of ICAM-1 up-regulation. Various flavonoids were incubated with β-NF overnight, followed by treatment with B[a]P. Only flavonoids that contained a C-ring hydroxyl substitution and a B-ring double bond were able to protect against ICAM-1 induction by B[a]P measured by flow cytometry.
This study suggests that B[a]P is able to increase endothelial cell adhesiveness by increasing ICAM-1, but only when activated by AhR-dependent enzymes and that this effect can be protected against by pre-treatment with selective flavonoids. (Supported by grants from NIEHS, NIH (P42ES07380), AHA predoctoral fellowship (0615216B), and the UK AES)

2221 THE ROLE OF OXIDATIVE STRESS IN THE DIFFERENTIAL EFFECTS BETWEEN N-3 AND N-6 PUFAS AND THE PROTECTIVE EFFECTS OF ALGAL AND FISH OIL IN COLON CARCINOGENESIS.

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Fish, rich in n-3 PUFAs, is claimed to give protection among others against colon cancer. Other PUFA sources like marine micro-algae are considered to replace fish. The question is whether PUFAs derived from micro-algae are a good alternative regarding chemoprevention in the colon. The mechanism by which n-3 PUFAs reduce cancer risk was studied, in particular the role of oxidative stress. In vitro studies showed no difference between algal and fish oil. Non-hydrolyzed algal oils and fish oil had a much lower effect on viability and cell proliferation of Caco-2 cells than hydrolyzed oils and the free fatty acids EPA and DHA. Oxidative stress was shown to play a role in the antiproliferative properties of EPA and DHA, as vitamin E partially reversed their effects. In a reporter gene study the n-3 PUFAs EPA and DHA appeared to be effective inducers of EpRE-regulated genes, whereas the n-6 PUFA arachidonic acid (AA) was not. These n-3 PUFAs need to be oxidized to induce EpRE-regulated gene expression, as vitamin E could partially inhibit their effect. After 8 weeks of dietary treatment, azoxymethane-induced colonic aberrant crypt foci (ACF) were scored in male Fischer 344 rats. The incidence of ACF was significantly lower in rats fed fish or algal oil diets compared to rats fed corn oil diet. No difference between the results from the fish and algal oil diets were found. When analyzed with Gene Set Enrichment Analysis, data from microarrays showed a significant induction of both the antioxidant genes and the xenobiotic detoxification enzymes pathway. The results support a similar mode of action of algal and fish oil. Induction of EpRE-regulated genes may be a pathway by which n-3 PUFAs, in contrast to n-6 PUFAs, are anticarcinogenic.

2222 TOXICOLOGICAL EVALUATION OF RESVIDA™, A HIGH PURITY *TRANS*-RESVERATROL.

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trans-Resveratrol is a naturally-occurring, polyphenolic compound found predominantly in grapes. There has been growing interest in its dietary use because of reported beneficial properties, including antioxidant, anti-inflammatory, anti-carcinogenic and anti aging activities. However, dietary supplements and food ingredients must clearly show safety-in-use. To that end, the safety of the proprietary high purity *trans*-resveratrol; Resvida™, has been investigated, amongst others, in subchronic and genotoxicity studies. In a 13-Week oral study in Wistar rats Resvida™ was administered in the diet at 0, 120, 300, and 750 mg Resvida™/kg bw/day. A slight decrease in food consumption and body weight gain was observed in males and females treated at 300 and 750 mg Resvida™/kg bw/day; however these observations were due to palatability and were not considered to be adverse effects. Due to palatability effects, the effective high dose was approximately 700 mg/kg bw/day and this dose was defined as the no-observed-adverse-effect-level (NOAEL) for Resvida™. Analysis of blood samples revealed that plasma concentrations of free and conjugated *trans*-resveratrol did not increase during the 13-Week treatment period in any dose group, indicating that no accumulation occurs in plasma. Urinalysis, hematological and histopathological analysis at Week 13 revealed no significant treatment-related effects. Resvida™ was non-mutagenic in a standard Ames test using *Salmonella typhimurium* strains TA98, TA 100, TA1535, and TA1537, and *Escherichia coli* strain WPZ uvrA (pkM101) at concentrations up

to 5000 µg/plate with and without metabolic activation. In a chromosomal abberation test, Resvida™ exhibited clastogenic activity in human lymphocytes at 10, 20 and 30 µg/ml without metabolic activation and at 40 and 50 µg/ml with metabolic activation. However, in an *in vivo* bone marrow micronucleus test in rats, Resvida™ was non-genotoxic at a dose up to 2000 mg/kg bw/day. Taken together, these studies provide evidence that Resvida™ has an overall low order of toxicity.

2223 IMPLICATION OF MODE OF ACTION (MOA) FOR RISK ASSESSMENT OF CATECHIN-RICH BEVERAGES.

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Sponsor: J. Avalos.

Catechins, which are polyphenol compounds found in abundance in green tea, have been of high interest due to their beneficial effects on health. Focusing on the beneficial effects, catechin-rich beverages have been marketed in Japan as Food Specified for Health Use.
Earlier we demonstrated that green tea extract used for the catechin-rich beverages showed clastogencitiy effect *in vitro* while it was not confirmed in *in vivo* studies (Toxicologist vol.96, Abstract No.1441). We also reported that the generation of hydrogen peroxide (H2O2) was responsible for the *in vitro* clastogenicity.
Since then, we explored factors that contributed to the generation of H2O2 to understand if clastogenicity demonstrated *in vitro* may pose an actual risk when people ingest the catechin-rich beverages. Substantial amounts of H2O2 were generated when catechins were incubated under *in vitro* culture conditions. However, interestingly, extremely low amount of H2O2 was detected when incubated in water at the same concentration. Further investigation revealed that generation of H2O2 is steeply increased when pH was above 6. This indicates that pH is a key factor in determining how much H2O2 is generated via catechins.
Polyphenols including catechins are known to act as antioxidant due to its reducing potential. However, under *in vitro* culture conditions, catechins mainly act as prooxidant by reducing ambient or dissolved oxygen to form H2O2. Analysis of each catechin revealed that generation of H2O2 is well correlated with its reducing potential supporting this scheme. Measurement and/or calculation of H2O2 under the conditions when catechins are ingested in a beverage indicated that actual exposure of H2O2 to humans is relatively non-existent.
Regarding the clastogenicity potential and based on the understanding of mode of actions (MoA), we concluded that there is no appreciable impact on health risk following intake of the catechin-rich beverages.

2224 MICROARRAY ANALYSES OF VOMITOXIN-INDUCED CHANGES IN P53 HETEROZYGOUS AND WILD-TYPE MICE: COMPARISION OF GENE EXPRESSION CHANGES WITH CLINICAL AND HISTOPATHOLOGIC MARKERS OF TOXICITY.

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A series of chronic toxicity studies were undertaken to characterize the responses of transgenic p53+/- (TG) and corresponding wild-type (WT) mice to a battery of carcinogenic and non-carcinogenic fungal toxins, including non-carcinogenic vomitoxin (deoxynivalenol). Mice were exposed to 0, 1, 5 or 10 ppm vomitoxin in diet for 26 weeks. Vomitoxin caused a significant dose-dependent reduction in body weight in WT and TG mice, accompanied by declining liver fat stores. A significant dose-dependent trend towards increased plasma total IgA levels in WT mice was consistent with the known effects of vomitoxin on the immune system. In TG mice plasma immunoglobulin levels were not affected. Microarray analyses of kidney tissues indicated that 42 genes, many associated with cellular metabolism, were differentially expressed in control versus vomitoxin-treated WT mice. Forty-eight genes were differentially expressed in kidneys from control and vomitoxin-treated TG mice, including more genes related to transcription and transport than were affected in WT mice. Real-time PCR analyses indicated that kidney cyclin D and cyclin E expression declined in vomitoxin-treated WT and TG mice. Overall, the effects of 26-week vomitoxin exposure on WT and TG mice were consistent with those previously seen in B6C3F1 mice exposed to vomitoxin for two years. Microarray and PCR analyses support the conclusion that vomitoxin is non-carcinogenic. Pathway analyses of microarray data from WT and TG mice will provide insight into the role of the p53 gene in vomitoxin-mediated toxicity. The results underscore the utility of microarray approaches in support of toxicologic assessment in the context of chronic toxicity studies or cancer bioassays.

2225 OPTIMIZATION OF A NEW PROCEDURE FOR THE SIMULTANEOUS DETERMINATION OF LINCOMYCIN AND SPECTINOMYCIN BY LIQUID CROMATOGRAPHY-TRIPLE QUADRUPOLE-MASS SPECTROMETRY IN FOOD.

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Spectinomycin and lincomycin are used in veterinary medicine for the treatment of infectious processes because their association provides better effectiveness that separated. In spite of their combined utilization, there is only an available analytical method proposed for the determination of both compounds in liquid hog manure samples (Peru et al., J. Chromatogr. A 1107: 152; 2006). The aim of this work is to develop an analytical strategy for determining spectinomycin and lincomycin using pressurized liquid extraction (PLE) and LC–MS/MS with a triple quadrupole (QqQ) mass analyzer. For quantification in milk and feed food samples a PLE procedure, using methanol/water mixture (80:20) was employed. The separation was carried out on a column Phenomenex Luna C18 (150 x 4.6 mm I.D., 5 µm) protected by a guard column C18 (4 x 2 mm I.D.) using a gradient of methanol/water in heptafluorobutyric acid 1 mM. Electrospray ion source (ESI) operating in the positive ion (PI) mode, coupled with LC conditions was optimized. Calibration curves showed excellent linearity, with satisfactory correlation coefficients (r2 >0.99) for the two antibiotics in the range of 0.005 to 100 mg ml-1. The recoveries obtained were 90.4% and 80.5%, precision, expressed as relative standard deviation (RSD) were 12% and 17% and limits of quantification (LOQs) were 0.08 mg ml-1 and 0.15 mg ml-1 for lincomycin and spectinomycin, respectively. PLE coupled with LC-ESI-MS/MS enables rapid and accurate determination of spectinomycin and lincomycin in whole, semi and skimmed milk and in feed food at lower levels than the established LMRs. This work has been supported by project No. AGL2006-04438, Ministry of Science and Technology, Spain.

2226 EFFECT OF N-3 AND N-6 POLYUNSATURATED FATTY ACID CONSUMPTION ON DEOXYNIVALENOL-INDUCED TISSUE-SPECIFIC IL-6 EXPRESSION IN MICE.

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Although fatty acids have long been proposed to modulate immunity and inflammation, the mechanisms remain poorly understood. The purpose of this study was to determine the comparative effects of feeding diets rich in various unsaturated fatty acids on induction of IL-6 by the trichothecene mycotoxin deoxynivalenol (DON). Female B6C3F1 mice were divided into 3 groups and fed daily for 8 wk a modified AIN-93G diet consisting of 1% corn oil and 6% of high-oleic safflower oil (SAF [n-9]), DHA enriched fish oil (DHA [n-3]), or corn oil (CRN [n-6]). Mice were then orally gavaged with either DON (10 mg/kg body weight) or saline vehicle. After 2 h, blood was collected, mice sacrificed, and tissues excised (liver, spleen, lung, and kidney) and analyzed by either IL-6 ELISA (serum) or quantitative RT-PCR analysis (tissues). Oral DON exposure substantially increased serum IL-6 concentration and IL-6 mRNA expression in all tissue samples. Splenic IL-6 mRNA expression was significantly influenced by diet ($p<0.05$), with SAF and DHA groups exhibiting 63% and 34% of that observed for CRN, respectively. A similar trend was observed in liver, with SAF and DHA groups showing 81% and 77% of that observed for CRN, respectively. Lung and kidney IL-6 mRNA responses to DON were not affected by PUFA in the diet. These data indicate that DON can induce IL-6 expression in multiple organs and that polyunsaturated fatty acids can selectively exacerbate or attenuate this response in an organ-specific manner.

2227 THE FUNGAL METABOLITE, PYRROCIDINE A, INDUCES APOPTOSIS IN HEPG2 HEPATOCYTES AND PK15 RENAL CELLS.

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Pyrrocidines are polyketide-amino acid-derived antibiotics produced by Acremonium zeae, a prevalent seed-borne endophyte of corn. Pyrrocidines exhibit potent activity against Gram-positive bacteria, including drug resistant strains, and display significant activity against Candida albicans, as well as fumonisin and alfa-

toxin producing fungi. We have shown that pyrrocidines A and B are cytotoxic in two mammalian cell lines, HepG2 cells and PK15 cells, with pyrrocidine A more potent than pyrrocidine B. Morphologic changes were consistent with apoptosis. In this study we examined the caspase-8 (an initiator in the extrinsic/receptor-mediated apoptotic pathway), caspase-9 (an initiator in the intrinsic apoptotic pathway) and caspase-3/7 activity (effectors of apoptosis) as well as the amount of released lactate dehydrogenase (LDH; compromised cell membrane integrity) and MTT formazan formed in the mitochondria (viability) at 1, 4 and 24 hours after addition of 25 µg pyrrocidine A /mL to cultured HepG2 and PK15 cells. Pyrrocidine A treatment caused an early decrease in mitochondria activity and activation of caspase-8/9 followed by activation of caspase-3/7 and LDH release in both cell types. These findings indicate that pyrrocidine A induced cytotoxicity is the result of apoptosis initiated through both the intrinsic and the receptor mediated apoptotic pathways. Given the toxicity of pyrrocidines in mammalian systems, in vivo studies are essential for risk assessment.

2228 SAFETY ASSESSMENT OF THE SOYBEAN ACETOLACTATE SYNTHASE PROTEIN (GM-HRA).

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Expression of the acetolactate synthase protein (GM-HRA) in genetically modified (GM) soybeans confers tolerance to herbicidal active ingredients of the sulfonylurea and imidazolinone classes through the introduction of two specific amino acid changes. Here we report the safety assessment results of the GM-HRA protein. Bioinformatic comparison of the amino acid sequence of GM-HRA did not identify similarities to known allergenic or toxic proteins. *In vitro* digestibility studies demonstrated that the GM-HRA protein was rapidly degraded in simulated gastric fluid containing pepsin (<30 sec) and in simulated intestinal fluid containing pancreatin (<1 min). The enzymatic activity was completely inactivated at 50°C for 15 minutes demonstrating that it is heat labile. The GM-HRA protein expressed *in planta* is not glycosylated, and soybeans containing the GM-HRA protein produced similar protein/allergen profiles without significant changes in total protein or individual proteins/allergens as its non-transgenic counterpart. No evidence of adverse effects were observed in mice following acute oral exposure to the GM-HRA protein or in a 28-day repeated dose dietary toxicity study at doses up to 1000 mg/kg/day. This comprehensive assessment demonstrates the safety of the GM-HRA protein when used in the context of agricultural biotechnology.

2229 SAFETY ASSESSMENT OF GLYPHOSATE ACETYLTRANSFERASE (GAT4601) PROTEIN.

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A *glyphosate acetyltransferase* (*gat*) gene from *Bacillus licheniformis* was subjected to multiple rounds of gene shuffling to optimize kinetics of corresponding GAT proteins to acetylate glyphosate. Genetically modified soybeans expressing the *gat4601* gene (356043 soybeans) are tolerant to glyphosate. Here, we report the safety assessment results of the GAT4601 protein. Bioinformatic comparison of the amino acid sequence of GAT4601 did not identify similarities to known allergenic or toxic proteins. *In vitro* digestibility studies demonstrated that the GAT4601 protein was rapidly degraded in simulated gastric fluid containing pepsin (<30 sec) and in simulated intestinal fluid containing pancreatin (<2 min). The enzymatic activity was completely lost at temperatures above 56°C demonstrating that it is heat labile. The GAT4601 protein expressed *in planta* is not glycosylated, and soybeans containing the GAT4601 protein produced similar protein/allergen profiles without significant changes in total protein or individual proteins/allergens as its non-transgenic counterpart. No evidence of adverse effects were observed in mice following acute oral exposure to 2000 mg/kg of GAT4601 protein or in a repeated dose dietary exposure study at doses of 800-1000 mg/kg/day. This comprehensive assessment demonstrates the safety of the GAT4601 protein when used in the context of agricultural biotechnology.

2230 SUBCHRONIC DIETARY TOXICITY OF HIGH OLEIC SOYBEAN EVENT DP-3Ø5423-1 NUTRITIONALLY-MODIFIED USING GENE SUPPRESSION TECHNOLOGY.

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A 90-day rat feeding study was conducted as part of the regulatory safety assessment of genetically-modified (GM) soybean event DP-3Ø5423-1 containing both the *gm-fad2-1* gene fragment and the *gm-hra* gene. Genomic insertion of the *gm-fad2-1* gene fragment suppresses expression of the endogenous FAD2-1 gene encoding the seed-specific Ω-6 fatty acid desaturase, resulting in a high-oleic phenotype. GM-HRA, a modified soybean acetolactate synthase (ALS), resists ALS-inhibiting herbicides. Diets nutritionally comparable to PMI® Certified Rodent LabDiet® 5002 were formulated by substitution of processed soybean fractions from 305423, non-GM isoline control, or 3 non-GM commercial reference soybean varieties at a concentration (by wt) of approximately 20% dehulled/defatted toasted meal, 1.45% toasted ground hulls, and 1.6% degummed, alkaline-refined oil. All diets were fed to young adult Crl:CD(SD) rats (12/sex/group) for at least 95 days. Compared with rats fed the non-GM control diet, no biologically relevant differences were observed in rats fed the 305423 diet with respect to body weight/gain, food consumption/efficiency, mortality, clinical signs of toxicity, or ophthalmological observations. No test diet-related effects were observed on neurobehavioral assessments, organ weights, or clinical or anatomic pathology. Response variable values for animals in the test diet group fell within the range of biological variation observed for animals in the reference diet groups. Under the conditions of this study, daily consumption of a rodent diet containing meal, hulls, and oil from soybean 305423 caused no adverse health effects in young adult rats. We conclude that high oleic soybean event DP-3Ø5423-1 is nutritionally equivalent to and as safe as non-GM soybeans.

2231 LOW DOSE DIETARY GENISTEIN NEGATES THE THERAPEUTIC EFFECT OF TAMOXIFEN.

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Breast cancer is the most commonly diagnosed cancer in women. 75% of breast cancers occur in postmenopausal women and the majority of these are estrogen dependent, which respond to antiestrogen like tamoxifen. It is observed that the dietary isoflavone supplements have been consumed by more American women, including the postmenopausal women with breast cancer by assuming that soy intake would be a safe alternative to hormone replacement therapy. Genistein, a predominant isoflavone in soy, acts as an estrogen agonist at physiologically relevant dosages and has shown to stimulate growth of estrogen-responsive breast cancer cells. If the breast cancer patients with tamoxifen treatment consume considerable isoflavone supplements, the potential combined effect of tamoxifen and genistein may result in significant undesirable outcome on mammary tumors.

In this study, we investigated interaction of three dietary dosages of genistein with tamoxifen on the growth of estrogen-dependent breast cancer (MCF-7) cells implanted in ovariectomized athymic mice. We found that 250ppm and 500ppm genistein induced mammary tumor growth over the inhibitory effect of tamoxifen in a dose dependent manner. However at higher dosage, 1000ppm genistein did not negate the effect of tamoxifen. It is important to note that high incidence of tumor was observed in the 1000ppm treatment group. The high rate of apoptosis in this group may explain the small tumor size. Mice tumor characterization using primary culture indicates these tumors retain the estrogen dependent phenotype. In summary, the interaction of genistein with tamoxifen is complex, involving multiple mechanisms depending on dosage.

2232 SAFETY AND EFFICACY EVALUATION OF PEDIOCOCCUS ACIDILACTICI AS AN ORAL PROBIOTIC.

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A short-term study of the safety and efficacy of oral administration of Pediococcus acidilactici to male Sprague Dawley rats was conducted to assess potential use as a probiotic supplement. Groups of ten rats received doses of 0, 1 X 10*9, 2 X 10*9 or 10 X 10*9 cfu once per day for 15 days. No differences in body weight or food consumption were observed among groups. Fecal samples were collected daily to assess

bacterial viability. At the completion of the study, blood was collected for evaluation of complete blood counts and serum parameters including cholesterol, albumin, total bilirubin, alanine amino transferase, alkaline phosphatase, aspartate aminotransferase, and gamma glutamyl transferase. No lesions were observed during gross necropsies or upon evaluation of histological samples of liver, heart, spleen, kidneys, stomach, small intestines or colon. Rats consuming the probiotic demonstrated effects on various immune parameters including lymphocyte subpopulations and lymphocyte proliferative responses to Con A and LPS, but not on natural killer cell activity. These results support the safety in use and potential benefits of us of Pediococcus acidilactici as a probiotic supplement.

2233 SUBCHRONIC DIETARY TOXICITY OF SOYBEAN EVENT DP-356Ø43-5 GENETICALLY-MODIFIED USING A NOVEL GENE SHUFFLING TECHNOLOGY.

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A 90-day rat feeding study was conducted as part of the regulatory safety assessment of genetically-modified (GM) soybean event DP-356Ø43-5 expressing the *gat4601* and *gm-hra* genes (Optimum™GAT™, registered trademark of Pioneer Hi-Bred). Event 356043, tolerant to multiple classes of weed-management herbicides, is the first food crop produced using a novel gene-shuffling technology. Acetyltransferase (AT) gene sequences from 3 strains of *B. licheniformis* were recombined *in vitro* to create a functionally-optimized enzyme, GAT4601, that inactivates the herbicidal active ingredient glyphosate. GM-HRA, a modified soybean acetolactate synthase (ALS), resists ALS-inhibiting herbicides. Diets nutritionally comparable to PMI® Certified Rodent LabDiet® 5002 were formulated by substitution of processed soybean fractions from 356043, 356043 treated in-field with glyphosate and sulfonylurea herbicides, the non-GM isoline control, or 3 non-GM commercial reference soybean varieties at a concentration (by wt) of 20% dehulled/defatted toasted meal and 1.5% toasted ground hulls. All diets were fed to young adult Crl:CD(SD) rats (12/sex/group) for at least 92 days. Compared with rats fed the non-GM control diet, no biologically relevant differences were observed in rats fed either of the 356043 diets with respect to body weight/gain, food consumption/efficiency, mortality, clinical signs of toxicity, or ophthalmological observations. No test diet-related effects were observed on neurobehavioral assessments, organ weights, or clinical or anatomic pathology. Response variable values for animals in the test diet groups fell within the range of biological variation observed for animals in the reference diet groups. Under the conditions of this study, daily consumption of rodent diets containing meal and hulls from 356043 soybean caused no adverse health effects in young adult rats. We conclude that Optimum™GAT™ soybean is nutritionally equivalent to and as safe as non-GM soybeans.

2234 NON-CARIOGENICITY OF HYDROGENATED POLYDEXTROSE, A SYNTHETIC LOW-CALORIE POLYSACCHARIDE SUGAR REPLACER IN FOODS.

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Polydextrose is a water-soluble, low-calorie (1 kcal/g) glucose polymer that has been widely used in foods since 1981. Its functional effects include the bulking of non-nutritive sweeteners to mimic the mouthfeel and other organoleptic properties associated with standard mono- and disaccharide sugars. Hydrogenated polydextrose is a novel, reduced form of polydextrose. Hydrogenated polydextrose meets Food Chemicals Codex and JECFA specifications and is approved by the US FDA (21 CFR 172.841), Codex Alimentarius and the food regulatory agencies of 31 other countries, for use in food.

Several human studies utilizing in situ intraoral plaque pH telemetry devices show that confections made with hydrogenated polydextrose do not cause the oral microflora-driven decrease in pH associated with the development of human dental caries. The studies described herein examined hard and soft confections containing 17-88% hydrogenated polydextrose. The test confections gave lowest intraoral plaque pH values of 5.8 – 6.4 after 30 minutes of chewing vs 4.3 – 4.5 for the 10% sucrose positive control solution. Non-plaque oral cavity measurements of erosive potential gave acid flux values of 1.5-6.4 mol H+ /min/liter for the test confections vs 40 mol H+ /min/liter for the positive sucrose control.

As a consequence, hydrogenated polydextrose meets FDA's criterion for a noncariogenicity health claim (21 CFR 101.80), i.e., when used in a finished food to completely replace sugars, it does not lower intraoral plaque pH below 5.7 either during or up to 30 minutes after consumption.

2235 RAW264.7 MACROPHAGE CELLS - AN *IN VITRO* ALTERNATIVE FOOD SAFETY TESTING PLATFORM COMPATIBLE WITH VARIOUS FOOD TYPES AND FOOD-BORNE TOXINS.

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The public's demand for safer foods creates incentive for manufacturers to explore alternative food production methods to reduce or eliminate food-borne hazards. Quantitative measures of toxin and pathogen removal are then needed to compare the efficacies of alternative food processing strategies. Sensitive instrumental, immunochemical, and bacterial assay methods are available for this purpose although some are selective for a single toxin or pathogen or single categories of related agents. Furthermore, chemical and immunochemical assay methods do not normally report biologic activity. Food processing may introduce structural modifications of toxic agents that interfere with quantification. Yet, in some cases, the modified toxin may possess toxic activity. Thus, assays that detect biological activity are natural complements for the sensitive methods of analysis alluded to above.

We report the utility of cultured RAW264.7 macrophage cells as a general testing platform for evaluating the biological activities of toxic agents of relevance to food safety and defense. In our series of cytotoxicity studies we found that RAW264.7 macrophage are compatible with a spectrum of food types in liquid (infant formulas, apple juices, and orange juices), semisolid (yogurt and peanut butter), and solid form (wet and dry pet food). We observed that RAW264.7 cells are sensitive to typical cytotoxins such as ricin, abrin, Shiga toxin, fumonisin B1, and heavy metals. Interestingly, RAW264.7 cells, unlike cultured ACHN renal carcinoma or COLO205 colon carcinoma cells, are mildly sensitive to melamine and melamine cyanurate, agents associated the 2007 pet food recall.

Continuing studies are on-going to evaluate range of food compatibility and toxin sensitivity for RAW264.7 cells with the expectation that its broad application could reduce the need to use laboratory animals for routine testing.

2236 DIETARY FLAVONOIDS SULFATION BY HUMAN SULFOTRANSFERASES.

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Phase II drug metabolizing enzymes, cytosolic sulfotransferases (SULTs), catalyze the sulfation of many hormones, neurotransmitters, drugs, and xenobiotics. SULT1A1 is a simple phenol-preferring SULT, SULT1A3 is a monoamine-preferring SULT, SULT1E1 plays a major role in estrogen sulfation, and SULT2A1 is important for dehydroepiandrosterone regulation. Dietary flavonoids have been described as health-promoting and disease-preventing bioactive food compounds. In this study, dietary flavonoids, catechin, epicatechin, eriodictyol and hesperetin were investigated for their ability to act as substrates and inhibitors of human SULTs. The purified recombinant SULTs and human intestine cytosols were used as the source of enzyme. The results indicated that recombinant hSULT1A1 and hSULT1A3 as well as human intestine cytosol can catalyze the sulfation of the flavonoids investigated, and hSULT1A3 exhibit higher activity. The sulfation of catechin, epicatechin, eriodictyol and hesperetin by recombinant SULTs showed substrate inhibition at high concentrations of flavonoids. The sulfation of catechin and epicatechin catalyzed by human intestine cytosol did not show substrate inhibition in the concentration range used. The sulfation efficacy of flavanones, hesperetin and eriodictyol, is greater than that of flavanols, catechin and epicatechin. Our result also indicated that hesperetin and eriodictyol are potent inhibitors of purified hSULT1A1, hSULT1A3, hSULT1E1, and hSULT2A1. Catechin and epicatechin significantly inhibit hSULT1A1 and hSULT1A3, but they are not inhibitors of purified hSULT1E1 and hSULT2A1. Hesperetin and eriodictyol are more potent inhibitors than catechin and epicatechin. The sulfation efficacy and potency of inhibition is related to the flavonoids structure in C-ring. These results suggest that dietary flavonoids can be metabolized by SULTs. They may also regulate human SULT activities and therefore affect the regulation of hormones and neurotransmitters, detoxification of drugs, and the bioactivation of procarcinogens and promutagens.

2237 SAFETY ASSESSMENT OF LACTERMIN®, A WHEY EXTRACT.

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Milk has long been a reliable source of nutrients and as a feedstock for manufacture of dairy products. Whey, a protein rich by-product of cheese production, was regarded as having little value other than as a source of protein for manufac-

ture of other foods or as a nutrient supplement. More recently, whey and especially the basic whey proteins, have been found to possess a variety of beneficial activities including, but not limited to effects on immunity, anti-bacterial properties and repair of damaged gastrointestinal tissues. The proteins thought to be responsible for these the majority of these activities are identified as "growth factors". Lactermin® is a whey fraction produced by a proprietary process and includes lactoperoxidase and lactoferrin (comprising >50% of total protein) and, a variety of minor proteins and peptides including the growth factors, IGF-I, IGF-II, PDGF, FGF, TGF-beta and betacellulin, a member of the epidermal growth factor family. In anticipation of future use of Lactermin® in human and animal applications, a battery of in vitro and in vivo studies were conducted to determine its safety in use. In vitro, Lactermin® did not show any mutagenic properties in the Ames assay at up to 5000 μg/plate (w/ or w/out S9) and was likewise negative in the mouse lymphoma thymidine kinase assay at concentrations up to 5000 μg/ml. In vivo, Lactermin® was negative in the mouse micronucleus test at a maximum dose of 2000 mg/kg. In a 13 week feeding study, 10 rats per sex were fed Lactermin® mixed with the diet to deliver doses of 300, 1000 and 3000 mg/kg/day, neither sex exhibited any test article related clinical observations or effects on body weight, food consumption, ophthalmic effects, functional observational battery, organ weights, locomotor activity, hematology, serum chemistry, urinalysis or macroscopic or microscopic pathology. The results from the genotoxicity studies and the subchronic oral toxicity study suggest Lactermin® is safe for consumption with a no-observed-effect level (NOEL) of 3000 mg/kg/day.

2238 AFLATOXIN EXPOSURE DECREASES SERUM LEVELS OF VITAMINS A & E IN GHANAIANS AT HIGH RISK FOR AFLATOXICOSIS.

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Aflatoxin (AF) exposure results in decreased levels of serum and tissue vitamins (Vits) A & E in animals. Supplementing the diets of animals with Vits A & E suppressed AFB1-induced carcinogenesis. However, little information is available on human populations at high-risk for aflatoxicosis. In this study, AF exposure and Vits A & E levels were initially evaluated in a total of 507 Ghanaians. Serum AFB1-albumin adducts (SAFAA) were measured by radioimmunoassay and Vits A and E were determined by HPLC. The average level of SAFAA was 0.94±0.64 (range:0.1-4.44) pmol/mg alb.; the average level of Vit A was 1.32±0.48 (range:0.41-4.85) μmol/L, and the average level of Vit E level was 15.68±4.12 (range:6.35-30.40) μmol/L. A significant negative correlation was found between serum SAFAA and Vit A levels (r= -0.110, p=0.013). A more significant correlation was found between SAFAA and Vit E levels (r= -0.149, p<0.001). SAFFA levels were significantly higher in subjects who had lower levels for both Vits A & E (median:0.985 pmol/mg alb.) as compared to subject with higher Vits A & E levels (median:0.741 pmol/mg alb., ptrend = 0.001). To verify these findings, blood samples were re-collected in 165 subjects out of 507 people at 3 months after the initial collection. Significant negative correlations were confirmed between levels of SAFFA and both Vits A (r= -0.232, p=0.003) and E (r= -0.178, p=0.023). Again, higher SAFFA levels were found in subjects with lower levels of Vits A & E (median: 1.578 pmol/mg alb.) compared to subjects with higher levels of Vits A & E (median:1.381 pmol/mg alb., ptrend =0.002). These data suggests that AF exposure decreased levels of serum Vits A & E in high-risk human populations, which may have a significant impact on AF-related adverse health effects. (Supported by USAID LAG-G-00-96-90013-00).

2239 SEX DIFFERENCES IN APPARENT ADAPTATION TO IMMUNOTOXICITY OF DEOXYNIVALENOL.

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Deoxynivalenol (DON) is a mycotoxin produced by Fusarium graminearum and F. culmorum commonly found in grains. We hypothesized that DON was immunotoxic in BALB/c mice, suppressing peripheral blood lymphocytes at 1 ppm but not at lesser doses. Groups of 10 female and 10 male BALB/c mice were fed DON at 0, 0.25, 0.5, 1, and 2 ppm over 14 days and 28 days. Peripheral blood and single spleen cells suspension were stained with fluorescently labeled antibodies for CD4, CD19, CD8a and CD11b leukocyte cell surface markers. Flow cytometry was used to detect leukocyte phenotypes. In peripheral blood, the percentage of T cytotoxic and B cells were inhibited in both sexes of BALB/c mice after 14 days of DON exposure, and toxic dose of DON varied by sex, whereas exposure to DON over 28 days did not inhibit these lymphocytes, compared with the control diet. Dietary DON did not influence hematology in males but red blood cells (RBC) at 0.5 and 1 ppm DON and hemoglobin (Hb) at all DON doses were suppressed in female mice by dietary DON over 14 days. The inhibition of RBCs by DON disappeared after 28 days compared with the control diet. The percentage of monocytes (CD11b+) was decreased in peripheral blood (at doses of 0.5-2 ppm DON) and spleen (2 ppm DON) only in BALB/c female mice after 28 days compared with control diet. These results indicate that BALB/c mice adapted to most signs of DON immunotoxicity and hematotoxicity after 28 days. At this time, the percentage of monocytes was decreased in peripheral blood and spleen by as little as 0.5 ppm DON in female mice, suggesting that female sex hormones potentiate DON immunotoxicity in BALB/c mice. Supported by US Wheat Barley Scab Initiative.

2240 FUMONISIN B1 EXPOSURE PHENOCOPIES CHOLESTEROL DEPRIVATION IN C. ELEGANS.

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Fumonisin B1 is a mycotoxin that induces neural tube defects in mice and epidemiological studies suggest that it is a risk factor for neural tube defects in humans. In C. elegans, exposure to fumonisin resulted in no apparent phenotype, but their progeny were unable to properly shed their cuticle. This phenotype mimics cholesterol deprivation both in its generational delay and its molting phenotype. To better understand the molecular events underlying Fumonisin B1 toxicity, we performed microarrays using RNA isolated from the progeny of fumonisin-exposed C. elegans. C. elegans were exposed to 10 μM fumonisin B1 beginning at the L1 larval stage and continuing for 72 hours, by which point these C. elegans were adults. Fumonisin B1 exposure was then ended and progeny of exposed C. elegans were grown in the absence of fumonisin for an additional 72 hours and collected as young adults. RNA from these animals was then used to perform 6 (3-biological x 2-technical replicates) whole genome Agilent microarrays. We identified 554 up-regulated and 242 down-regulated genes, using a 1.5-fold change in expression and a p-value < 0.001. Gene Ontology analysis of differentially regulated genes, determined that there is a very high enrichment in collagen genes. Using Cytoscape to elucidate the interactions of differentially regulated genes, we found a network of interacting genes that centers on nhr-23, which is critical for molting in C. elegans. Merris et al. (J. Lipid Res. 48(5): 1159-1166) performed microarrays using C. elegans that were grown with low cholesterol levels and found an up-regulation of collagen genes and a down-regulation of nhr-23. Thus, fumonisin B1 exposure appeared to mimic cholesterol deprivation both in its phenotype and changes in gene expression.

2241 THE PI3K/AKT SIGNALING PATHWAY MEDIATES THE INDUCTION OF CHOLESTEROL BIOSYNTHESIS GENES BY α-CHACONINE IN INTESTINAL EPITHELIAL CELLS.

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The glycoalkaloid α-chaconine is a natural toxin found in potatoes (*Solanum tuberosum L.*). Although the toxicological effects of glycoalkaloids are well described, the mechanism(s) underlying these effects are poorly understood. We previously demonstrated by DNA microarray analysis that non-cytotoxic concentrations of α-chaconine induced expression of cholesterol biosynthesis genes in the intestinal epithelial Caco-2 cell line. In addition, the expression of genes involved in MAPK and PI3K/AKT signaling pathways and genes encoding downstream effectors of these pathways such as c-Jun, FOXO3a, Myc and Fos was found to be affected.
In the present study, we examined whether the induction of cholesterol biosynthesis genes by α-chaconine is mediated by MAPK and PI3K/AKT pathways. To that end, we first determined whether α-chaconine induced phosphorylation of MAPK (ERK, JNK, p38) and AKT kinases in differentiated Caco-2 cells. In a subsequent experiment, cells were pretreated with either LY294002 (60 μM) or PD98059 (50 μM) or SP600125 (60 μM) for 24 hours to block phosphorylation of AKT, ERK

and JNK respectively, prior to coincubation with 10 μM α-chaconine for 6 hours. Real time RT-PCR experiments were conducted on selected cholesterol biosynthesis genes to determine the effect of inhibition. We observed that α-chaconine induced significant phosphorylation of AKT, ERK and JNK but not of p38. Only inhibition of phosphorylation of AKT was able to abrogate the induction of cholesterol biosynthesis genes. Therefore, the main conclusion from the study was that activation of AKT and not ERK or JNK is necessary for induction of cholesterol biosynthesis genes by α-chaconine.

2242 HIGH CONTENT DNA DAMAGE ASSAY: SIMULTANEOUS MEASUREMENT OF H2AX PHOSPHORYLATION, ATM ACTIVATION, AND DNA CONTENT COMBINED WITH LIVE CELL ANALYSIS.

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We developed a high-content assay that allows characterization of various forms of genomic and cellular injury. First, live adherent cells are stained with a cocktail of Hoechst 33342 to label DNA, Mitoshift to monitor mitochondrial functionality, YoPro to monitor cell membrane integrity, and propidium iodide to measure late stages of cell death. Second, cells were fixed and stained with antibodies to pATM-Ser1981 (Alexa 633) and pH2AX (Alexa 488), measures of early response to DNA damage. Cells were counterstained DAPI. A panel of reference compounds with known effects on DNA damage, including topo I and II inhibitors, and ROS scavengers and donors were evaluated. Short compound exposure time (pulse labeling) experiments were effective in elucidating cell cycle-related effects. Using the topo.I inhibitor CPT as an example, cells entering into S-phase presented pATM and pH2AX as distinct foci, reporting the number of DSBs per nucleus as well as the kinetics of their formation. At higher DNA damage levels, the foci were too dense for individual enumeration. Instead, the overall IF integrated over the nucleus was a successful measure of pH2AX activation. With longer incubations (approximately one doubling time) the effects on cell proliferation and blockage in phases of the cell cycle were analyzed. Interesting results include those obtained with the anti-oxidant N-acetyl cystine showed a decrease in H2AX-related foci at low dosages, but beginning at a critical dosage (3.13 uM), a single two-fold dosage increase (to 6.26 uM) caused extensive DSB breakage, followed by loss of membrane integrity and mitochondrial function at the next highest dosage (12.52 uM), indicating toxicological effects.LSC allows the combination of data acquisition and analysis from live cells and fixed cells to add greater depth to high-content toxicological screens and assays.

2243 TRACING MICRONUCLEI BY FLUORESCENT LIVE CELL IMAGING ANALYSIS.

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It is believed that micronuclei (MN) originate from chromosome fragments or whole chromosomes that lag behinds at anaphase during cell division. However, nobody looked its emergence in a living cell and nobody knows its consequence after cell division. Advance in chemical and microscopic technologies offers the resolution. Developments in fluorescent dyes and proteins have facilitated the study of complex cellular processes using fluorescent staining or labeling of various proteins, ions and lipids in living cells. A fluorescent microscope designed for live observation enable to visualize cellular dynamics played by fluorescent organelles and proteins without cell killing. Using the live cell imaging technology, we traced the emergence of MN and its consequence in living cells to further understand cellular mechanisms of MN formation. Human lymphoblastoid TK6 are very proliferating cells and are commonly used for in vitro MN tests. We developed dual-color fluorescent TK6 cells in which histon H3 and tubulin were differentially expressed as fusion to red (mCherry) and green (EGFP) proteins, respectively. Under fluorescent microscopic observation, nuclei (red) and microtubules (green) in the living cells can be visible. The parental TK6 and the derived fluorescent clone had similar proliferating rate, suggesting that expression of these proteins does not affect cell-cycle progression. Using computer-controlled con-focal fluorescent microscope system designed for live cell imaging, we recorded time-lapse, dual-color images of nuclei (chromosome) and microtubules in the living cells. Because this system can reconstruct the images of nuclear division by 3-dimension, we never miss the emergence of MN even when it generates behind the main nuclei.

2244 *IN VITRO* MICRONUCLEI SCORED BY FLOW CYTOMETRY: AN INTERNATIONAL, MULTI-LABORATORY TRIAL.

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A multi-lab trial was conducted to evaluate a flow cytometry (FCM) method for scoring micronuclei (MN) in L5178Y cells. Participating labs were supplied with prototype In Vitro MicroFlow kits, and each was assigned 1 genotoxicant, (etoposide, mitomycin C, or vinblastine) and 1 non-genotoxicant (sucrose, dexamethasone, or staurosporine). The reference lab (Litron) performed experiments with each of the 6 agents. Cells were treated for 24hrs over a range of concentrations up to 5 mg/ml, or overtly cytotoxic concentrations. MN were scored via standard microscopy and FCM. In addition to MN counts, an FCM-based cytotoxicity measurement was evaluated (relative survival based on "counting beads"). Key findings include the following: (1) significant discrepancies in top concentration selection were found when cytotoxicity measurements were based on different assays, with the counting bead approach tending to be the most sensitive. (2) Both microscopy- and FCM-based scoring methods detected concentration-dependent MN formation for the three genotoxic agents studied, with good agreement between the reference and the participating labs. (3) Whereas FCM and microscopic analyses showed no significant increases for the non-genotoxicants when top concentration was based on counting beads, significantly elevated MN frequencies were observed for concentrations that were chosen based on other cytotoxicity endpoints. Our results indicate that rapid assessment of genotoxicity can be accomplished with a simple FCM method, and that this technique is highly transferable. Also, the concurrent assessment of cytotoxicity that is accomplished by FCM may help reduce the occurrence of irrelevant positive results, as it may represent a better means for choosing top concentrations.

2245 TIME-RELATED PROFILES OF MICRONUCLEATED PCES AND NCES IN BOTH BLOOD AND BONE MARROW AS MEASURED BY MICROSCOPY AND FLOW CYTOMETRY.

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Detection of micronuclei in both peripheral blood and bone marrow is accepted by regulatory guidelines. A micronucleus is defined as a chromosome and/or fragment left in the cytoplasm during erythrocyte maturation. Micronucleated polychromatic erythrocytes (MNPCE) are pre-mature cells in bone marrow and micronucleated normochromatic erythrocytes (MNNCE) are mature cells in peripheral blood. In this study, the dynamics of MNPCE and MNNCE formation and persistence were evaluated in CD-1 mice treated with cyclophosphamide (CP: clastogen) or colchicine (Col: aneugen). At 24, 48, and 72 hrs post treatments, bone marrow and blood were collected for analysis. Flow cytometric analysis of these samples was performed at Litron Laboratories, while standard microscopy techniques were utilized at Covance Laboratories. MNPCE, MNNCE, and PCE/NCE ratios in both blood and bone marrow were scored by both methods. Based on slide evaluation, MNPCEs in bone marrow reached a maximum at 24 hrs after CP and Col treatments, and then gradually declined to background level by 72 hrs. MNPCEs in blood increased at 48 hrs after the treatments and reached their high levels at 72 hrs. Increases in MNNCEs in the blood correlated well with increases in MNPCEs observed in the bone marrow at 48 and 72 hrs. Conversely, MNNCEs in blood did not correlate with MNNCEs observed in bone marrow at any of the time points examined. Higher levels of MNPCEs and MNNCEs were observed in the cytotoxic range (%PCE < 20) at 48 and 72 hrs. The flow cytometry analysis correlated well with the microscope method for the detection of MNPCEs and for evaluating cytotoxicity in bone marrow and blood.

2246 AUTOMATED FLOW CYTOMETRIC DETECTION OF MURINE PERIPHERAL BLOOD MICRONUCLEATED ERYTHROCYTES USING NO-WASH, ROOM-TEMPERATURE GTOX FLOW KIT.

E. Gautherot, F. Gaille, M. Herbert, A. van Agthoven, F. Malergue, F. A. Montero and J. Wolfers. *Beckman Coulter Immunotech, Marseille, France.* Sponsor: J. Allen.

Rationale & Scope: The mouse erythrocyte micronucleus test is one of a number of tests that identify substances that cause cytogenetic damage. This test measures micronucleated polychromatic erythrocytes (MN-PCE) in the blood stream. The

classical microscopy-based test requires counting DNA-containing reticulocytes, making it relatively time-consuming and labor intensive. Furthermore, due to the relative paucity of MN-PCE's, it is sometimes technically difficult to analyze sufficient data to permit the desired analysis of statistical significance, possibly resulting in lower sensitivity. Recently, flow cytometry has been proposed as an alternative approach to microscopy. This technique permits analysis of large numbers of relevant cells, leading to a gain in sensitivity as well as reduced time to results. However, flow cytometry is often implemented as a not-fully-automated technique that requires extensive technical expertise as well as manual sample preparation. This abstract describes a simple, quick, and fully automated sample preparation and flow cytometric approach that yields first results in less than 2 hours. Results: Sample preparation is performed at room temperature, without centrifugation steps, and can be run on the Biomek NXP sample preparation workstation. Flow cytometry data are automatically processed using barcode sample identification. Analysis of MN-PCE during validation studies shows high sensitivity (<0.2% MN-PCE in non-treated animals) and excellent reproducibility (CV=20% for non-treated mice and 10% for treated mice). Finally, comparison of this method with existing flow cytometry methods for both non-treated mice and mice treated with MMS, MMC, cytosine and vincristine shows extremely high correlation coefficients (R2 > 0.9). Conclusion: This fully automated mouse peripheral blood erythrocyte micronucleus application enhances laboratory throughput and provides results comparable to those obtained with other flow cytometry methods.

2247 DEVELOPMENT OF AUTOMATED *IN VITRO* MICRONUCLEUS AND CYTOTOXICITY SCREENING ASSAYS.

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Expensive late stage failure would be avoided if toxic compounds were identified earlier in high throughput and cost effective manner. High content analysis (HCA) is able to quickly analyze hundreds of compounds using objective image analysis software rather than manual visual scoring. Using HCA approach, we have developed two formats for automated in vitro micronucleus mammalian assay: mononucleated and binucleated micronucleus assays. We used GE Healthcare's IN Cell Analyzer 1000 digital image analysis platform for an objective, quantitative and rapid evaluation of compound effects on MN formation in 384-well microplates. In addition, we multiplexed MN assay with an apoptosis marker, activated Caspase-3, to measure simultaneously MN formation and apoptosis induction on the same cells and to eliminate false positives by excluding apoptotic cells from MN assays analysis. Overall, both assays are powerful, reproducible tools for genotoxicity analysis. However, our results suggest that the mononucleated format is superior to the binucleated format in continuously growing cell lines. A multiplex approach that generates apoptosis data provides added value for the in vitro micronucleus assay. The adaptation of the in vitro micronucleus assay to automated and accelerated throughput imaging platform makes it the best choice for genotoxicity testing in early drug development.

2248 ANEUGENIC COMPOUNDS INCREASE MICRONUCLEUS FREQUENCIES IN MONONUCLEATED CELLS IN THE CB MICRONUCLEUS TEST.

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The micronucleus assay in human lymphocytes was developed as a short term screening test for the detection of both clastogenic and aneugenic chemicals. For human lymphocytes it is recommended to score micronuclei by the cytokinesis block (CB) method using cytochalasin B. The original method developed by Fenech and Morley, 1985, focusses exclusively on binucleated cells. However, recent studies suggest that micronuclei in mononucleated cells could provide complementary information. Results obtained with aneugenic compounds show a dose-dependent increase of micronuclei in mononucleated cells. At present, the underlying mechanism has not been clearly indentified. In order to obtain more information two immunofluorescence techniques were employed involving CREST analysis for detection of kinetochore proteins and staining of phosphorylated histone H2AX (gH2AX). The CREST analysis reveals whether micronuclei in mononucleated cells contain chromosomal fragments or whole chromosomes. The gH2AX staining detects phosphorylation of histone H2AX at serine 139 rapidly occurring at sites flanking DNA double strand breaks. Our results suggest that micronuclei in mononucleated cells can be used to investigate the aneugenic activity of chemicals in a fast and easy way, and can be included in the CB assay with human lymphocytes.

2249 USING KINASE SELECTIVITY PROFILES TO PREDICT *IN VITRO* MICRONUCLEUS ASSAY RESULTS.

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Inhibition of kinases involved with chromosome segregation during mitosis can cause aneuploidy and lead to a positive micronucleus assay (MNT) result. Because most small molecule kinase inhibitors (SMKIs) target the conserved ATP binding pocket, many SMKIs are not selective and are prone to non-pharmacologically related toxicities. Internal experience has demonstrated that a proportion of Roche SMKIs test positive in an in vitro MNT, yet our understanding of the kinases mediating this toxicity is limited. To better understand this relationship, a statistical model was built correlating in vitro MNT results with inhibition profiles against 290 kinases, using a training set of 54 chemically diverse SMKIs. The model was developed in two steps: first, a pair of feature selection techniques were used to identify kinases most correlated with MNT results; and second, support vector machines, was used to generate a model based on the subset of selected kinases. Cross validation was used to estimate the accuracy of the model and to determine the optimal kinase set: The model's prediction accuracy was estimated to be 80% and the optimal set of features was comprised of 13 kinases, including CDK2, GSK3α & β, and PCTK1 & 2. Subsequently, the model has been applied to an independent 19 compound forward validation set where it properly predicted the MNT results for all 19 compounds (100%). Further validation of the model includes testing of 40 additional SMKIs in the MNT assay, results which will be completed by the end of the year. siRNA knock-down experiments of the 13 kinases are also being conducted to further understand their role in chromosomal segregation during mitosis. Although it is anticipated that this model will not remain perfect following additional validation tests, the strong correlation of kinase inhibition profiles with MNT results has proven to be of benefit for intelligent medicinal chemistry design.

2250 AUTOMATED *IN VITRO* MICRONUCLEUS TESTING OF NATURAL COMPOUNDS IN CORRELATION WITH HYDROGEN PEROXIDE DETECTION.

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The in vitro micronucleus test (MNT) is a well-accepted technique for assessing the clastogenic/aneugenic potential of compounds. In a high content screening (HCS) approach we measure micronucleus induction in CHO-K1 cells. The fully automated evaluation of micronuclei is based on fluorescent image analysis. Similar to other mammalian genotoxicity tests, the in vitro MNT is characterized by a low specificity, meaning that this assay is prone to false-positive results. Natural, antioxidative compounds like polyphenols have been shown to become prooxidative under certain culture conditions leading to the formation of peroxides, which may result in a positive in vitro MNT. This effect is an in vitro artifact and does not occur in vivo.

The aim of the present study was to evaluate a set of natural compounds with regard to their genotoxicity and to correlate this potential with the formation of hydrogen peroxide in cell culture medium. A sensitive colorimetric assay was used for the detection of hydrogen peroxide with epigallocatechin gallate (EGCG) as a positive control.

The micronucleus frequency induced by different concentrations of EGCG correlated well with the amount of hydrogen peroxide found in the medium. Interestingly, other natural compounds that were also positive in the MNT, were negative for peroxide formation. Further, a third group was identified of being negative in the MNT, although chemical structure and antioxidative potential suggest positive results due to prooxidative action. Thus, distinct structural properties seem to be responsible for the potential of natural antioxidative compounds to yield a positive in vitro MNT. Apart from the formation of hydrogen peroxide in the cell culture medium, other mechanisms play a role. Along with the growing interest in the properties of bioactive phytochemicals, it is essential to further investigate mechanisms of direct or indirect interaction with the DNA in order to interpret positive in vitro genotoxicity tests.

2251 VALIDATION AND OPTIMIZATION OF THE IN VITRO MICRONUCLEUS ASSAY IN HUMAN LYMPHOCYTES.

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We previously reported validation of the *in vitro* micronucleus (MN) assay in human peripheral blood lymphocytes (HPBLs) using the clastogens mitomycin C (MMC) and cyclophosphamide (CP), and comparing whole blood (WB) cultures

vs. isolated lymphocytes (IL). Since these earlier studies did not reveal any appreciable difference in response between WB and IL, subsequent studies were performed with only WB to assess the responses for vinblastine (VIN) and colchicine (COL), known aneugens. Ten mL cultures (containing 0.6 mL WB) were initiated (at t = 0 hr) in 15-mL centrifuge tubes in RPMI 1640 containing 2% phytohemagglutinin M, and grown at 37°C and 5% CO2. Cultures were treated (at t = 24 hr) for 3 hr with and without S9, and for 20 hr without S9 only. Concentrations evaluated were 10.0 - 40.0 ng/mL COL and 12.5 - 50.0 ng/mL VIN. Water was the solvent control. Treatment was ended by centrifugation and washing in media, and the cultures were re-incubated in fresh media. Cytochalasin B (cytB) was added (6 μg/mL, final; at t = 45 hr) and the cultures were harvested at 72 hr. Slides were stained with Giemsa and May-Grunwald, and 200 cells/culture were scored for cytotoxicity (cytB blocked proliferation index, CBPI), and 400 binucleated cells were scored for the frequency of MN (%MN BN). Dose-dependent decreases in CBPI were not observed for either COL or VIN. As anticipated, no binucleates were found following the extended treatment with COL -S9. Statistically significant and/or dose-dependent increases in %MN-BN were observed for all treatments in both experiments. Although the increases did not reach significant levels in the second trial for COL +S9, the increases were dose dependent, and *bona fide* positive responses were observed in the concurrent cultures treated S9. These experiments indicate that COL and VIN are suitable positive controls under the conditions evaluated. Additional studies are in progress to determine the minimal culture size that can be used to decrease compound requirements without sacrificing sensitivity.

2252 AN IMPROVED FLOW CYTOMETRY-BASED UNSCHEDULED DNA SYNTHESIS (UDS) ASSAY.

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Potential genotoxicity of new chemicals and drugs, and increased regulatory and industry concern, have driven the need for more reliable, rapid, high-throughput genotoxicity tests. The UDS assay is an established method to measure DNA damage caused by genotoxins and UV radiation via detection of radiolabelled nucleotides incorporated into repaired DNA. To replace the use of animals and radioactive materials, we have developed a Flow Cytometry-based in vitro UDS assay (FLUDS). The FLUDS assay measures biotin-dUTP incorporation during DNA repair. Our original FLUDS assay was limited by a narrow dynamic range and low signal to noise ratio, which also limited its accuracy. To improve this method, we introduced an incubation of the cell cultures with aphidicolin (Aph), a DNA repair enzyme (polymerase alpha) inhibitor, to allow accumulation of DNA strand breaks before the biotin-dUTP uptake/repair step and subsequent fluorescent anti-biotin antibody detection by flow cytometry. Cells were irradiated with UVC (0 or 20 J/m^2) in the presence and absence of 100 μM Aph. Treatment with No UV, UVC, No UVC+Aph, and UV+Aph resulted in %UDS+ cells of 2.6%, 4.7%, 2.5% and 68.0%, respectively. This corresponds to Test/Control (T/C) ratios of 1.8 for cells not treated with Aph, and 27.2 for cells treated with Aph. Thus, the signal/background ratio increased approximately 15-fold in UVC irradiated cells when Aph was present. Cells were also treated with methyl-methanesulfonate (0 or 250 μM MMS), a direct-acting genotoxin, with and without Aph. Treatment with Vehicle Control, MMS, Vehicle +Aph, and MMS+Aph resulted in %UDS+ cells of 0.7%, 0.9%, 1.5% and 11.3%, respectively. T/C ratios were 1.3 and 7.5 for cells treated with and without Aph (a 5.8-fold increase). Overall, the use of Aph enhanced both UDS assay sensitivity and accuracy. Our optimization of the FLUDS assay has made high-throughput UDS tests feasible, while eliminating animal use and providing a more objective, quantitative genotoxicity assay.

2253 HIGH CONTENT SCREENING CELL-BASED ASSAYS FOR *IN VITRO* GENOTOXICITY MEASUREMENTS.

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Genotoxicity is a significant factor in the failure of drugs during pre-clinical development. Genotoxicity can be caused by damage to DNA by several agents including environmental toxicants, topoisomerase inhibitors and oxidative stress. The toxicity is manifested as DNA single (SSB) and double strand (DSB) breaks. DNA damage induced check point signaling plays an important role in maintaining genomic integrity in cells. Phospho ATM and phospho H2AX sense and transduce the DNA damage signal to Chk2 which along with activated ATM phosphorylate p53 at ser15 and 20. The stabilized p53 then leads to cell cycle arrest at G1/S or G2/M allowing the damage to be repaired. Ku 70 and 80 are dimeric proteins involved in DNA double strand break repair. Cellular profiling of these markers using DNA damage agents and ranking them for their potency is a powerful tool to study genotoxicity of compounds. High Content Screening (HCS) assays which involve automated, quantitative cell based imaging, enable multiplexed measurements of the DNA damage markers in individual cells and cell populations. We developed a panel of quantitative HCS assays for Phospho ATM, p53, Phospho Chk2 (Thr68), Ku 70/80, which enabled easy evaluation of time- and dose-dependent genotoxic responses of different compounds in cells in an automated high throughput format. Using this panel of assays to evaluate several compounds including etoposide, camptothecin, cisplatin and paraquat, we ranked them for their genotoxic potency. This study shows that screening for DNA damage markers using high content screening allows automated, fast and quantitative evaluation of many compounds for genotoxicity in individual cells & cell populations.

2254 GENOTOXICITY SCREENING STEPS UP A GEAR: VALIDATION OF HIGHER THROUGHPUT PROTOCOLS FOR THE GADD45A-GFP GENOTOXICITY ASSAY (GREENSCREEN HC).

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The GreenScreen HC genotoxicity screening assay exploits the proper regulation of the GADD45a gene to drive GFP accumulation in human (TK6) cells. Data analysis from an expanding set of compounds (>300) consistently reveals high sensitivity for genotoxic carcinogens (>80%), without compromising high specificity (>90%), i.e. the discrimination of non-genotoxins.
Drug developers require the early and accurate identification of potentially toxic compounds to enable either rejection, or chemical modification to optimise lead quality. In previous GreenScreen validation studies, testing has been performed over 9 dilutions, in triplicate and often with range finding trials. In order to use the assay in early high throughput screening, fewer dilutions and replicates are tolerated, and the top concentration is limited by the toxicity of DMSO. This work tested the sustainability of high sensitivity and specificity when the assay was restricted in this way, and has significantly expanded the data set to examine the sensitivity of GreenScreen to more diverse chemical classes.
1266 compounds from the Sigma-Aldrich Library of Pharmacologically Active Compounds have been tested, each at 3 serial dilutions from 100 μM in 96 well plates. This is a practical limit for screening samples stored at 10 mM in 100% DMSO, and is the lowest concentration that has been proposed for regulatory testing. It is closer to a physiologically relevant dose level. The rate of positive results was 7% for genotoxicity and 33% for cytotoxicity. Analysis using DEREK for Windows toxicity prediction software demonstrated a high correlation of positive results with alerts for chromosome damage, mutagenicity and carcinogenicity. Of 25 LOPAC compounds that were duplicated in the standard protocol, 2 expected positives were negative, as a consequence of the reduced top concentration.
Preliminary work increasing the assay plate density to 384 and 1536 wells for ultra-high throughput screening will also be presented.

2255 ASSESSMENT OF THE GENOTOXICITY OF METABOLITES GENERATED BY HEPATOCYTES AND S9 EXTRACTS USING THE GADD45A-GFP GREENSCREEN HC ASSAY.

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The assessment of metabolites is an essential part of genotoxicity testing. Whilst hepatocytes were successfully used many years ago as an exogenous metabolite generating system, their supply was not reliable, so instead 'S9' liver extracts were adopted to supplement the *in vitro* assays. Genetic toxicologists are currently preoccupied with concerns about low specificity in the *in vitro* mammalian genotoxicity tests, and this has also stimulated the International Workshop on Genotoxicity Tests (IWGT) to revisit metabolic considerations. An IWGT Strategy Expert Group recently drew attention to the limited capacity of S9 to accurately predict *in vivo* metabolism. Technological advances now allow the reliable supply of stabilised, metabolically active hepatocytes. At the same time, an ever increasing data set from the new human cell, GADD45a-GFP genotoxicity assay (GreenScreen HC) has proved it to have a consistently higher specificity than the current *in vitro* genotoxicity tests. The original, spectrophotometric GADD45-GFP assay could not be used to assess the genotoxicity of S9 metabolites; however this has now been made possible with the use flow cytometry. Data to demonstrate this will be presented. We have subsequently been able to assess metabolites by combining cells from the reporter assay with stabilised rat hepatocytes (from Abcellute Ltd). Again flow cytometry data to demonstrate this will be presented. We shall report the results of an ongoing comparative study in which a selection of over 40 non-proprietary genotoxins, pro-genotoxins, and non-genotoxins are being assessed with rat S9 extracts and hepatocytes as alternative sources of exogenous metabolism in the GADD45a-GFP assay. Data from the study will be presented, comparing the sensitivities and specificities of the different approaches.

2256 INDEPENDENT VALIDATION OF THE GREENSCREEN GADD45-ALPHA INDICATOR ASSAY USING ONE HUNDRED PROPRIETARY AND NON-PROPRIETARY COMPOUNDS.

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The GreenScreen GADD45α indicator assay has been reported to have high concordance with regulatory genotoxicity as well as rodent carcinogenicity test data. Interestingly, the assay discriminated properly amongst compounds that tested positive in in vitro mammalian chromosomal damage assays identified to be either rodent carcinogens or non-carcinogens, indicating that the assay distinguishes between true- and false-positive in vitro mammalian results. One hundred proprietary and non-proprietary compounds were screened in the assay in two phases to address its robustness, sensitivity, and specificity in a blinded and independent validation study. The first study phase was comprised of 20 structurally diverse Roche compounds, of which 11 were identified internally as genotoxic (MNT and/or Ames positive) and 9 as non-genotoxic. The non-proprietary compounds were comprised of 9 genotoxic carcinogens, 5 genotoxic non-carcinogens, 5 non-genotoxic carcinogens, and 10 non-genotoxic non-carcinogens. Concordance for the first study phase compounds with results from the established genotoxicity assays was 63% (sensitivity of 28% (7/25) and specificity of 100% (24/24)) whereas concordance with rodent carcinogenicity was 55% (sensitivity of 21% (3/14) and specificity of 87% (13/15)), with 33% of the genotoxic carcinogens properly being identified. Fourteen compounds in this validation set had previously been tested in the GreenScreen assay, thirteen of which had concordant results (93%), indicating that the assay is robust. Phase one results from this blinded, independent validation study indicate that the GreenScreen assay is a robust and highly specific genotoxicity assay, however, a lower level of sensitivity was seen than what was previously reported for detecting genotoxiciants or genotoxic carcinogens. Phase two testing of 50 additional compounds is underway, the results of which are expected in the near future and will be included in the final analyses when presented.

2257 UTILIZATION OF GENE EXPRESSION TECHNOLOGIES IN THE DEVELOPMENT OF A PREDICTIVE ASSAY FOR GENOTOXICITY.

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Evaluation of the genotoxicity of compounds is an important aspect of safety testing during drug discovery and development. The in vitro micronucleus assay is a common genotoxicity screening assay used to assess the potential for compounds to produce chromosomal aberrations and aneugenicity prior to nominating lead compounds. Manual scoring of the micronucleus assay is labor intensive and an alternative assay with higher throughput is desired. The goals of this study were to evaluate the feasibility of using p21/GADD45a mRNA signal change in the prediction of genotoxicity and to co-develop a hybrid p21/GADD45 promoter-driven luciferase reporter vector to be used in creating a stably transfected cell line that could be used for high-throughput screening of compounds for prediction of genotoxicity. To determine whether or not GADD45 and p21 mRNA levels correlate with micronucleus formation, MCF7 cells containing wild-type p53 and MCF7-E6 cells lacking functional p53 were treated with a battery of micronucleus-positive and –negative compounds. When RT-PCR data was compared to the published genotoxicity information for commercial compounds, GADD45a and p21 expression levels showed 90% and 80% concordance respectively. To increase the sensitivity and specificity of a genotoxicity assay utilizing measurement of p21 and GADD45 expression levels, DNA-damage responsive regions of these two promoters were individually cloned upstream of a constitutive SV40 promoter in a luciferase reporter vector. MCF7 and MCF7-E6 were transiently transfected with the reporter constructs and treated with various genotoxicants as well as non-genotoxic agents. Increased reporter gene activity following treatment with genotoxicants in these experiments confirm that both promoters are induced by genotoxic agents. In addition, this induction is occurring to a greater level in the MCF7-E6 cells than the wild-type MCF7cells suggesting that the response of GADD45 and p21 to genotoxicants may be occurring via a p53-independent mechanism.

2258 OPTIMIZING A MICROTITER PLATE VERSION OF THE YEAST DEL ASSAY FOR THE SCREENING OF CHEMICAL LIBRARIES.

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Advancements in combinatorial chemistry, molecular biology, genomics, and automation have enabled the identification of a relatively large number of compounds capable of reacting with intended pharmacological targets. A large percentage of these compounds fail during clinical development due to safety related issues. The overall attrition of drug candidates due to genetic toxicology issues alone includes approximately 12% of drug candidates. Thus, the ability to predict genetic safety of compounds at early stages of drug discovery using a fast and economical assay would decrease delays in the introduction of vital drugs to patients and cut economic losses.

A microtiter version of the yeast DEL assay has been established for the purpose of screening large chemical libraries [N. Hontzeas et al., Mut. Res., 2007 (In Press)]. The classic plate version of the assay measures DNA deletions and has identified animal carcinogens with a high degree of accuracy (92%) including Ames positive as well as negative carcinogens. In the current work, we are optimizing this high throughput assay for assessing the genotoxicity of compounds that cause DNA deletions. We also compare the results of the classic plate assay to those obtained with the HTS version.

2259 POSITIVE GENOTOXICITY RESULTS DUE TO THE FORMATION OF GENOTOXIC REACTION-PRODUCTS OF THE SOLVENT DMSO WITH CARBOXYLIC/SULFONIC ACID HALIDES.

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To investigate the influence of solvents like DMSO on the outcome of genotoxicity assays, several compounds from different chemical classes were tested by using different solvents in the Ames II assay, a liquid microtiter modification of the standard Ames plate incorporation test.

Most of the compounds from the two chemical classes carboxylic acid halides and sulfonic acid halides are alerted by the in silico tool DEREK (Deductive Estimation of Risk from Existing Knowledge) to give positive genotoxicity results and are described in the literature (NTP database...) as genotoxic compounds. However, in-house studies showed different results in the Ames II assay, depending on the solvent used. Positive results in the Ames II with and without metabolic activation in the Salmonella typhimurium tester strains TAMix and TA98 could be confirmed, when using DMSO as solvent. In contrast, the same compounds were tested negative in the Ames II assay, when using other solvents than DMSO like water or ethanol.

These results may be explained by different reactions of the compounds in the solvents. Carboxylic/sulfonic acid halides in water should completely react to the corresponding and non-genotoxic carboxylic/sulfonic acids and hydro halide acids. But when using DMSO as solvent, besides the corresponding carboxylic/sulfonic acids, dimethylsulfide halides are also formed as reaction products of DMSO. These dimethylsulfide halides are alkylating agents, which are more stable in aqueous conditions and more likely to react with DNA, resulting in a positive Ames II assay.

As a consequence of these results, it is recommended using other solvents than DMSO to test the genotoxic potential of the two chemical classes carboxylic acid halides and sulfonic acid halides, to avoid such false positive results from DMSO reaction products. Positive results using DMSO as solvent for carboxylic/sulfonic acid halides are considered not relevant for aqueous, physiological conditions.

2260 COMPARATIVE IN VITRO INHIBITION OF RAT HIPPOCAMPAL MONOACYLGLCYEROL LIPASE BY CHLORPYRIFOS OXON, PARAOXON AND DIISOPROPYLFLUOROPHOSPHATE.

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Endocannabinoids are chemical modulators produced from membrane lipids involved in the regulation of the release of acetylcholine and other neurotransmitters in the mammalian brain. These signaling molecules are degraded by the enzymes fatty acid amide hydrolase (FAAH) and monoacyl glycerol lipase (MAGL). Organophosphates (OPs) are anticholinesterases that elicit cholinergic toxicity by inhibiting acetylcholinesterase, with a consequent increase in synaptic acetylcholine levels. Moroever, some OPs have been reported to inhibit both FAAH and MAGL activities in whole mouse brain with differing potencies. Thus, cannabinergic-cholinergic interactions may have toxicological relevance in anticholinesterase intoxications. We evaluated the *in vitro* potency and efficacy of three OPs (chlorpyrifos oxon [CPO], paraoxon [PO] and diisopropylfluorophosphate [DFP]) towards rat hippocampal MAGL, the enzyme primarily responsible for inactivation of the endocannabinoid 2-arachindonyl glycerol. Both CPO and PO exhibited relatively similar efficacies against MAGL (60-65% inhibition), while DFP had somewhat lesser efficacy (50-52% maximal inhibition). Marked differences in potency were noted, with rank order being CPO>>PO≥DFP (IC_{50}, 10 min, 37°C: 0.15, 8.1 and

10.4 μM, respectively). OPs with potent inhibitory actions against endocannabinoid degrading enzymes could influence acetylcholine release/accumulation following extensive acetylcholinesterase inhibition, and thereby modulate cholinergic toxicity. Knowledge of these interacting signaling pathways could be important in the management of anticholinesterase intoxication and other disorders, e.g., Alzheimer's disease, that involve altered cholinergic neurotransmission. Supported by Howard Hughes Medical Institute (A.L., A.N.), Oklahoma State University Board of Regents, and NIEHS (R01 ES009119, C.N.P).

2261 COMPARISON OF ENZYME INHIBITION PATTERNS FOLLOWING DEVELOPMENTAL CHLORPYRIFOS EXPOSURE USING DIFFERENT ADMINISTRATION PARADIGMS.

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Different methods of administration can produce differences in the toxicokinetics (absorption, distribution, metabolism, excretion) of a chemical. In the literature, the most common methods of administration of the OP insecticide chlorpyrifos (CPS) in developmental neurotoxicity studies are either by subcutaneous injections in DMSO or by oral gavage in oil. It is thought that CPS administered subcutaneously in DMSO is rapidly and completely absorbed and evenly distributed throughout the body. However, an oral CPS exposure may provide greater opportunity for first-pass metabolism in the liver. Using cholinesterase (ChE) inhibition as a marker of exposure, these two paradigms were compared in 10 day old rat pups. CPS (5 mg/kg) was administered either by subcutaneous (SC) injection in DMSO or by oral gavage (PO) in corn oil and ChE was measured in the serum and brain at various times following exposure. Inhibition of serum ChE was detected in the PO rats earlier than in the SC rats but both progressed to a similar level of peak inhibition of 80-90% at 8 hrs. However, recovery of activity appeared to occur faster in the SC rats. In the brain, the pattern of inhibition was similar with peak inhibition occurring at 8 hrs with greater inhibition in the PO group. As with the serum, recovery of activity appeared to occur faster in the SC rats with greater inhibition still remaining in the PO rats at 24 hrs. These data suggest that subtle differences are present between the two paradigms.

2262 ENHANCED STARTLE RESPONSE IN ADULT ZEBRAFISH AFTER EARLY DEVELOPMENTAL CHLORPYRIFOS EXPOSURE: SENSITIVITY OF A RAPID AUTOMATED NEUROBEHAVIORAL TEST.

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Developmental exposure to the organophosphate pesticide chlorpyrifos (CPF) has been widely shown to cause persisting neurobehavioral impairments in rodent models. Zebrafish are a useful complementary model of developmental neurobehavioral toxicity. Previously, we found that 100 ng/ml of CPF in the water of developing zebrafish for the first five days after fertilization causes persisting impairment in spatial learning. Tactile startle response, a higher throughput test, was used in the current study. Adult zebrafish were placed individually in an array of eight Petri dishes. Swimming activity after a tactile tap administered by computer driven solenoids was assessed by a computerized video-tracking device. Ten consecutive trials (1/min) were run to determine startle response and its habituation. Recovery trials were run at 8, 32 and 128 minutes after the initial trial set. The log of the distance swum in the five seconds after the tap was the dependent measure. The CPF-exposed fish showed a significantly ($p<0.025$) greater overall tactile startle response during the 10-trial session compared to vehicle-treated controls. No significant difference was seen during the initial trial, but the effect emerged over the later trials as the controls rapidly habituated to the tap while the CPF-treated fish did so more slowly. During the initial recovery period 8 min after the session the CPF-exposed fish also showed a significantly ($p<0.01$) greater startle response than controls. This study shows that a quick automated test of a simple behavior can detect a persisting neurobehavioral impairment caused by developmental CPF exposure in zebrafish. This may be helpful in screening for persisting adverse neurobehavioral affects of a variety of toxicants.

2263 PRENATAL CHLORPYRIFOS EXPOSURE IN MICE IMPAIRS WORKING AND REFERENCE MEMORY IN FEMALES AS ASSESSED BY A NOVEL FORAGING MAZE.

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Exposure to organophosphate pesticides (OPs) at doses below the threshold for systemic toxicity, are associated with disruption of neural pathways responsible for optimal cognitive development. Behavioral deficiencies observed in rodents after chlorpyrifos (CPF) exposure model the subtle cognitive impairments CPF potentially has in humans. As previously seen in rats, prenatal CPF exposure impairs working and reference memory in mice. We administered doses below the threshold for overt toxicity (0, 1 or 5 mg/kg/day) of CPF, s.c. in DMSO, to pregnant mice daily on gestational days 17-20. We assessed cognitive performance of offspring in a foraging maze, which is similar to a radial-arm maze except that at the end of each arm there is a T-junction. Food reinforcements are placed in the left part of the T at the end of each arm. The maze evaluates working memory (radial arm choice) and reference memory (T-junction choice) simultaneously. Consistent with findings in rats, we found sex-selective CPF effects on both working and reference memory. Female mice exposed to 5 mg/kg/day CPF showed the most deficits in reference memory ($p< 0.01$) and working memory ($p<0.02$), compared to the lowest dose and control groups. In contrast, CPF exposed males showed no significant impairment. Like previous studies in rats, response latency was not significantly affected in either sex. Results also show that errors in working and reference memory in the foraging maze, which assesses memory errors simultaneously, compared to the radial arm maze, which measures memory errors individually, yield similar results. The ability of our foraging maze to accurately assess a combination of complex behavioral tasks after prenatal CPF exposure in mice shows that this assay is a precise tool to extrapolate results from rodent models to humans in order to predict adverse effects of CPF, and other OPs, on human cognition.
Support from Bundt Fund at UW Madison.

2264 CHLORPYRIFOS-OXON INTERFERES WITH AXONOGENESIS IN EMBRYONIC ZEBRAFISH.

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Axonal morphology is a critical determinant of neuronal connectivity, and perturbation of the rate or extent of axonal growth during development has been linked to behavioral deficits in animal models and in humans. We previously demonstrated that organophosphorus pesticides (OPs) inhibit axonal growth in primary neuronal cell cultures at concentrations that do not inhibit acetylcholinesterase (AChE), but data confirming that OPs similarly disrupt axon growth in vivo is lacking. In this study, we utilized transgenic zebrafish that express green fluorescent protein in dorsal root ganglia (DRG) sensory neurons, primary motoneurons or secondary motoneurons to determine whether chlorpyrifos (CPF) or its oxon metabolite (CPFO) alter normal spatiotemporal patterns of axon growth in vivo. Preliminary studies of zebrafish embryos exposed from 24-72 hr post fertilization to these OPs in the water at concentrations ranging from 0.004 to 3 μM, indicated that CPFO, but not CPF, inhibited AChE activity, suggesting that zebrafish embryos lack the metabolic enzymes necessary to activate CPF during this life stage. Exposure to CPF at these same concentrations also had no effect on axonal growth. However, CPFO significantly inhibited axon growth of secondary motoneurons, and this effect was observed at concentrations that did not inhibit AChE activity. CPFO also altered axonal growth of primary motoneurons and DRG sensory neurons but only at concentrations that also inhibited AChE. Inhibitory effects of CPFO on axon growth correlated with altered swimming behavior, suggesting that these structural changes had functional consequences. Our data suggest that in vitro cell-based models are predictive of toxicant effects on axonogenesis in vivo, and these findings support the use of zebrafish as a scientifically valid model for developmental neurotoxicity testing. These studies were supported by the Johns Hopkins University Center for Alternatives to Animal Testing.

2265 CHORPYRIFOS OXON-INDUCED CHANGES IN THIOFLAVIN T BINDING TO THE PERIPHERAL ANIONIC SITE OF HUMAN ACETYLCHOLINESTERASE.

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Previous work from this laboratory has shown that the capacities of certain anticholinesterase organophosphates, like chlorpyrifos oxon (*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphate), to inhibit acetylcholinesterase change as a function of inhibitor concentrations below about 100 nM. One possible explanation for this phenomenon is that these organophosphates interact with a secondary binding site on acetylcholinesterase, thereby modifying events at the active site so as to reduce the capacity of other oxon molecules to phosphorylate Ser- 203. Thioflavin t is a benzothiazole dye that increases its fluorescent signal when bound to the peripheral anionic site of acetylcholinesterase, and can therefore be used to evaluate putative binding of other compounds to this site. Human recombinant acetylcholinesterase (3 nM) was preincubated with chlopyrifos oxon at concentrations ranging from 100 – 500 nM for at least 10 min at room temperature in order to phosphorylate Ser-203 of all enzyme molecules. These samples were subsequently mixed with

thioflavin t ranging in concentrations from 100 – 10,000 nM, in a SX20 stopped flow spectrophotometer (Applied Photophysics, Leatherhead, UK). Thioflavin t fluorescence was measured with an excitation wavelength of 450 nM, and for emission a 475 nM cutoff filter was utilized. The dissociation constant, *Kd*, for the binding of thioflavin t to the peripheral anionic site was calculated by nonlinear regression analysis of the fluorescent signal versus ligand concentration. In the absence of chlorpyrifos oxon the thioflavin t *Kd* was 358 nM. Addition of chlorpyrifos oxon decreased the maximal fluorescence signal of thioflavin t by 30-35 % in a concentration-independent manner, while *Kd* continued to increase up to 489 % as the concentration of chorpyrifos oxon increased. These data suggest that phosphorylation of Ser-203 reduces the binding of thioflavin t at the peripheral anionic site, and that chlorpyrifos oxon also competitively displaces thioflavin t from that same site. (Supported by Grant ES012648 from NIEHS)

2266 EXPOSURE OF ZEBRAFISH (DANIO RERIO) EMBRYOS TO DELTAMETHRIN RESULTS IN ALTERATION OF DOPAMINERGIC GENE EXPRESSION AND INCREASED ACTIVITY.

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Because significant levels of pyrethroid pesticides, including deltamethrin, have been found in the urine of pregnant women, there is increasing concern that exposure to pyrethroids during early development may permanently damage the nervous system. Data from the Richardson laboratory demonstrate that mice exposed in utero to low levels of the pyrethroid pesticide deltamethrin exhibit elevated levels of the dopamine transporter (DAT) and increased locomotor activity. Increased expression of the transcription factors Nurr1 and Pitx3, which work in concert to influence proper dopamine neuron development, were also observed. Here, we sought to demonstrate that the the zebrafish embryo could serve as an alternative model to study the developmental neurotoxicity of deltamethrin. Embryonic zebrafish (3-4 hours post fertilization (hpf)) were exposed to deltramethrin (1ng/mL, 10 ng/mL, and 100ng/mL) and examined at 24 h intervals up to 120 hpf. The 100 ng/mL deltamethrin was lethal to the developing embryo, resulting in 98% mortality by 144 hpf. However, animals exposed to sublethal concentrations had increased swimming activity and uncontrolled movement. To determine whether deltamethrin exposure altered expression of the DAT, Nurr1 and Pitx3, quantitative RT-PCR was used. Exposure to deltamethrin (1 ng/mL) caused an increase in the expression of DAT at 24 hpf (6.5-fold) and 72 hpf (5 fold). Furthermore, expression of both Pitx3 and Nurr1 are also increased (24 hpf: pitx3- 6 fold; nurr1- 5 fold)(72 hpf: pitx3 4 fold; nurr1- 3.5 fold). These data demonstrate that deltamethrin exposure of the developing zebrafish embryo results in qualitatively similar effects as observed in our mouse model and suggest the zebrafish is an ideal alternative model for exploring the developmental neurotoxic effects of deltamethrin. Supported by NIEHS Grants R01ES015991 and P30ES005022

2267 DEVELOPMENTAL DELTAMETHRIN EXPOSURE PRODUCES IMPULSIVE-LIKE BEHAVIOR THAT IS REVERSED BY METHYLPHENIDATE: RELEVANCE TO ADHD.

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Attention-deficit hyperactivity disorder (ADHD) is characterized by impulsivity, hyperactivity, and attention deficits, all of which are thought to arise from catecholaminergic dysfunction. Recent data from this laboratory have shown that mice developmentally exposed to deltamethrin (DM) exhibit several features observed in children with ADHD. These include elevated dopamine transporter levels, hyperactivity, a paradoxical calming response to psychostimulants, and increased rate of responding under a fixed interval (FI) schedule of reinforcement. Because increased responding under an FI schedule has been shown to be a reliable predictor of impulsivity in children, we sought to determine whether developmental DM exposure produced impulsive-like behavior using a fixed ratio (FR) waiting for reward paradigm. Male and female offspring of mice exposed to either 0 or 3mg/kg DM throughout gestation and lactation were trained to lever press. The response requirement on the FR component was gradually incremented to 25. Once performance was stable, a wait component was added. After completion of an initial FR25, "free pellets" were delivered during the wait component at increasing delay intervals; intervening responses reset the FR requirement. DM exposure significantly increased response rates (males: 200%; females: 101%), FR resets (males: 167%; females: 103%), number of wait reinforcers (males: 38%; females: 28%) and significantly reduced wait time (males: 35%; females: 21%). Furthermore, male but not female DM mice emitted significantly more responses per reinforcer (53% and 20% respectively). Administration of the common ADHD therapeutic, methylphenidate (MPD) reversed DM-induced impulsive-like behavior in both genders. The parallels between mice developmentally exposed to DM and children with ADHD suggest that developmental DM exposure may be a risk factor for ADHD.
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2268 DELTAMETHRIN AND ESFENVALERATE INHIBIT SPONTANEOUS NETWORK ACTIVITY IN RAT CORTICAL NEURONS *IN VITRO*.

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Understanding pyrethroid actions on neuronal networks will help to establish a mode of action for these compounds, which is needed for cumulative risk decisions under the Food Quality Protection Act of 1996. However, pyrethroid effects on spontaneous activity in networks of interconnected neurons are not well described. Recently, it was demonstrated that deltamethrin inhibited spontaneous activity and decreased the burst duration of rat hippocampal neurons in vitro. The present study expands characterization of pyrethroid effects on network activity by examining deltamethrin (DM) and esfenvalerate (ESF) effects on spontaneous firing in networks of cortical neurons. Primary cultures of rat cortex were prepared from postnatal day 1 pups, and cells were plated onto multiple electrode arrays at a density of 200,000 cells/array. After 9-21 days in vitro, spontaneous network activity consisting of individual spikes and groups of spikes (bursts) developed and reached a stable level, at which time experiments were conducted. To limit the responses measured to glutamatergic networks, gabaergic input was blocked by addition of 10 μM bicuculline and 20 μM SCH 50911. These drugs increased the mean network firing (# of spikes/min) and bursting (#bursts/min) rates, as well as burst duration. DM or ESF stock solutions were prepared in 1:1 DMSO:ethanol, and were added at final concentrations between 10 nM and 10 μM. Both compounds caused a concentration-dependent inhibition of mean network spike and burst rates; at 10 μM, inhibition of mean spike rate exceeded 90% for DM and 80% for ESF. Both compounds also decreased burst duration in a concentration-dependent manner. Inhibition of mean spike rate by ESF was less potent (~3 μM; N= 2-6) than DM (~700 nM; N=6). These data demonstrate that pyrethroids inhibit spontaneous glutamatergic network activity in cortical neurons in a fashion similar to hippocampal neurons. (This is an abstract of a proposed presentation and does not necessarily reflect Agency Policy)

2269 MOLECULAR MECHANISMS OF DELTAMETHRIN-INDUCED ALTERATIONS IN DOPAMINE TRANSPORTER EXPRESSION.

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Previous studies have demonstrated that exposure of mice to the pyrethroid pesticide deltamethrin (DM) causes elevated levels of the dopamine transporter protein (DAT; Kirby et al., 1999; Elwan et al., 2005). However, the mechanism by which deltamethrin exerts its effects on the DAT remain unknown. In order to determine whether transcriptional changes were responsible for the elevated DAT protein levels, mice were dosed with 3 mg/kg DM by oral gavage on day 1, 8, and 15. Mice were sacrificed 1 day after the last dose and the midbrain was collected for determination of mRNA expression by Q-PCR. Exposure to DM resulted in a 40% increase of DAT mRNA levels. This was accompanied by increased expression of the nuclear transcription factors Nurr1 (35%) and Pitx3 (36%), which have been demonstrated to work in concert to regulate DAT expression. In vitro exposure of MES23.5 dopaminergic cells to various concentrations of DM for 24 hr produced similar effects on gene expression as the in vivo exposure. DM (10 nM) increased DAT, Nurr1, and Pitx3 mRNA expression by 34%, 71%, and 98% respectively. To determine whether interaction with sodium channels was responsible for these effects, cells were exposed to veratridine (10 nM), an alkaloid neurotoxin that delays closing of sodium channels in a manner similar to that described for DM. Veratridine exposure increased DAT, Nurr1, and Pitx3 mRNA expression by 77%, 41%, and 77%, respectively. Because delay of closing of sodium channels can result in persistent depolarization, we next determined whether depolarization of cells with a combination of KCl (10 mM) and CaCl2 (2 mM) would produce similar effects on gene expression. Depolarization increased expression of DAT, Nurr1, and Pitx3 by 50%, 46%, and 46%, respectively. Taken in concert, these data suggest that the interaction of DM with sodium channels leading to persistent depolarization and induction of genes that regulate DAT expression represents a plausible mechanism for DM's effects on the DAT.
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2270 EFFECT OF ORGANOPHOSPHATE ESTER INDUCED DELAYED NEUROPATHY (OPIDN) ON THE NEURONAL CELL BODY.

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OPIDN results in distal axonopathy progressing to myelinated nerve fiber degeneration. In this study we assess the effects of this process on the cell bodies of dorsal root ganglion neurons, a population of cells giving rise to affected fibers. For this purpose we revisited a previously described model of OPIDN in rats, one due to long-term exposure to two neurotoxic organophosphates in rats, in the presence of chronic stress (Jortner et al., Tox. Pathol. 33:378). Young adult male Long-Evans rats were administered the organophosphates over a 63-day period, with sacrifice on days 28, 63 and 90. The test compounds were tri-ortho-tolyl phosphate (TOTP) given by gavage at 75, 150 or 300 mg/kg on alternate days during the 14-28 and 49-63 day periods (14 total doses) and/or chlorpyrifos in two 60 mg/kg subcutaneous exposures (on days 7 and 42). Corticosterone was added to the drinking water at 400 micrograms/ml throughout the study, to model chronic stress. There was TOTP dose-related OPIDN, including the presence of inhibition of brain neurotoxic esterase and bilateral distal axonopathy progressing to myelinated fiber degeneration. The lesions were most marked on day 90, following the full course of treatment and a subsequent four-week period without exposure to organophosphates. Fiber lesions were seen in spinal cord gracile fasciculus, where there were many dystrophic axons, and in peripheral nerves. The sural nerve was prominently affected, and this included a diminished g ratio (axon diameter/fiber diameter) (Jortner et al., Brain Pathol. 16:S168). Chromatolytic or vacuolated dorsal root ganglion neurons were noted, but morphometric assessment (n= 3-6 rats/group, 8 to 38 cells histologically analyzed/animal) did not reveal significant treatment associated alteration of cell body area. Thus OPIDN induced by long-term toxicant exposure led to reactive changes in a population of neurons giving rise to affected fibers, without alteration in their cell body size. Supported by US-AMRMC DAMD17-99-1-9489.

2271 EFFECTS OF THE ORGANOCHLORINE PESTICIDE DIELDRIN IN THE NEUROENDOCRINE BRAIN OF LARGEMOUTH BASS (*Micropterus salmoides*).

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Exposure to the organochlorine pesticide dieldrin has previously been shown to disrupt steroidogenesis in fish which is stimulated by luteinizing hormone (LH) released from the pituitary. Because the neurotransmitter GABA stimulates LH release in fish, dieldrin may disrupt reproduction through direct effects on GABA signaling by antagonizing the GABA-A receptor. GABA receptors in the largemouth bass (LMB) brain were characterized using P2 membrane fractions from fresh LMB whole brain. Increasing concentrations (0-9.3 nM) of [35S]-t-butylbicyclo-phosphorothionate (TPBS), a GABA-A specific receptor ligand, were used to determine GABA receptor binding. The results suggest that there is a single TBPS binding site on GABA receptor, with a Kd of 0.39 nM and Bmax of 72.2 fmol/mg protein. Binding affinity of the LMB GABA-A receptor for dieldrin was determined by competition with [35S]-TBPS. To investigate in vivo effects of dieldrin, male LMB were injected with vehicle or a single dose of 10mg/kg dieldrin. Seven days after injection, the expression of glutamatic acid decarboxylase (GAD65, the enzyme that synthesizes GABA) and GABA-A receptor subunit gamma2, which is involved in modulation of receptor signaling, were quantified in the hypothalamus and telencephalon. Both GAD65 and GABA-A receptor subunit gamma2 mRNAs were induced approximately 3-fold in the telencephalon ($p<0.05$) but not in the hypothalamus, suggesting tissue specific regulation of these transcripts. The present study demonstrates that dieldrin has the potential to disrupt reproduction in LMB by affecting GABAergic signaling in the brain.

2272 CARBARYL-INDUCED CHOLINESTERASE INHIBITION AND DOSIMETRY IN YOUNG AND ADULT RATS.

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The N-methyl carbamate insecticides are a class of aceylcholinesterase (ChE) inhibitors that are regulated as a group by the US EPA. Data on the comparative sensitivity to ChE inhibition at different ages are necessary for risk assessments to be protective of infants and children. Systematic comparisons of neurotoxicity of this group of chemicals in young and adult animals are not available in the literature. To characterize the age-related differences in acute neurotoxicity to carbaryl, we evaluated the dose-response in adult, postnatal day (PND)17, and PND11 Long-Evans male rats. Tissues were taken 40 minutes (time of peak ChE inhibition) after oral dosing with either corn oil vehicle or carbaryl (3, 7.5, 15, or 30 mg/kg). ChE activity in brain and erythrocytes (RBC) was measured using a radiometric assay minimizing tissue dilutions. Brain and plasma were also analyzed for carbaryl levels using HPLC. The ChE activity data were modeled using benchmark dose (BMD) analyses to estimate a BMD10 (10% decrease in enzyme activity). The BMD10 for PND11 brain was half that of adults, i.e., the PND11 pups were about 2-fold more sensitive. However, the RBC BMDs were more similar, and their ratios were not significantly different from 1. Analysis of tissue carbaryl levels revealed dose-related increases of carbaryl in brain and plasma, but no significant age-related differences in the dose-response data were observed; however, the carbaryl levels were more variable than ChE activity. Evaluation of the ChE activity as a function of brain carbaryl levels showed that PND11 rats had slightly greater inhibition compared to either PND17 or adult at similar brain levels. RBC comparisons showed no such differences. Thus, brain ChE data revealed greater sensitivity at PND11, but not PND17, when compared to adults. This difference did not appear to be reflected as significantly greater levels of carbaryl in the brains of PND11 rats. This is an abstract of a proposed presentation and does not reflect US EPA policy.

2273 TIME COURSE OF THE TRANSCRIPTIONAL RESPONSE OF RAT CEREBROCORTICAL TISSUE AFTER ACUTE PYRETHROID EXPOSURE IN VIVO.

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Pyrethroid insecticides disrupt neuronal function by interfering with the function of voltage-sensitive Na+ channels (VSSCs). Distinct differences in the pharmacological actions of pyrethroid sub-types (Type I or Type II) on VSSCs have been observed. The impact of these pharmacological actions on downstream, intracellular signaling pathways has not been fully explored. This study tests if acute exposure to equipotent doses of Type I and Type II pyrethroids induce similar transcriptional changes in cerebrocortical tissue. Our goals are identification of biochemical markers of effect for the pyrethroids and comparison of effects across pyrethroid subtypes. Long-Evans rats were acutely exposed (p.o) to equipotent doses (ED30 for motor activity, mg/kg) of: deltamethrin (DLT), cypermethrin (CYP), cyfluthrin (CYF), permethrin (PERM), bifenthrin (BIF) or tefluthrin (TEF)(3,10.7,2.3,43,3.2 & 2.3 mg/kg, respectively, n=6/group). Tissue was collected at 3 or 6 h post-exposure with time-matched vehicle controls (n=12/group). Affymetrix Rat 230 2.0 GeneChips® were used to obtain transcriptional profiles. Two statistical methods, Linear Models for Microarray Data (limma) and two-way ANOVA with BH-multiple testing correction, were used to identify treatment related effects within each test compound. Expression of a number probe sets were altered for the Type II pyrethroids (DLT, CYP & CYF) with both the limma (102,82 & 127) and ANOVA (238,73 & 124) methods. A large degree of overlap in the altered probe sets was observed between Type II pyrethroids. No significant alterations in transcript expression were observed for the Type I pyrethroids (PERM, BIF & TEF) at these dose levels with either method. These data indicate that equipotent low-dose acute exposures to Type I and Type II pyrethroids do not produce equivalent genomic responses in rat cerebrocortical tissue. *This abstract does not necessarily reflect the policy of the US EPA.*

2274 ACTION OF PYRETHROID INSECTICIDES ON RAT Nav1.6 SODIUM CHANNELS EXPRESSED IN XENOPUS OOCYTES.

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Voltage-sensitive sodium channels are important sites of action for pyrethroid insecticides. Mammalian sodium channels are encoded by a family of 9 genes; native sodium channels are heteromeric complexes of one of these 9 α subunits and either one or two auxiliary (β) subunits. The Nav1.6 sodium channel isoform is widely expressed in the brain. We expressed Nav1.6 sodium channels, alone or in combination with the $\beta1$ and $\beta2$ auxiliary subunits, in Xenopus oocytes and evaluated the effects of the pyrethroid insecticides tefluthrin and deltamethrin using two-electrode voltage clamp. Tefluthrin (100 μM) produced clearly detectable resting modification of Nav1.6 sodium channels expressed in the absence of auxiliary subunits that was not altered by repetitive stimulation. Coexpression with the $\beta1$ subunit (Nav1.6+$\beta1$ channels) did not affect resting sensitivity to tefluthrin but conferred a 2.2-fold increase in sensitivity following repeated stimulation. Coexpression with the $\beta2$ subunit (Nav1.6+$\beta2$ channels) had no effect on tefluthrin sensitivity. Heterotrimeric (Nav1.6+$\beta1$+$\beta2$) channels exhibited sensitivity to resting and use-

dependent modification by tefluthrin that was nearly identical to that found for Nav1.6+β1 channels. In contrast resting modification of Nav1.6 channels, expressed either alone or with auxiliary subunits, by deltamethrin (100 μM) was barely detectable. However, modification of heteromeric complexes containing the β1 subunit (Nav1.6+β1 and Nav1.6+β1+β2 channels) by deltamethrin was enhanced 7.8- and 5-fold, respectively by repetitive depolarization. These results show that the sensitivity of Nav1.6 sodium channels to pyrethroids is strongly modulated by coexpression with the β1 subunit and that the relative importance of resting and use-dependent modification depends on the pyrethroid examined. Actions on brain sodium channels containing the Nav1.6 isoform may contribute to acute pyrethroid intoxication in mammals.

2275 LONG-TERM NEUROBEHAVIORAL CONSEQUENCES OF LOW-LEVEL NEONATAL PARATHION EXPOSURE IN RATS.

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Developmental exposure of rats to the organophosphate (OP) pesticides chlorpyrifos and diazinon has been found in our studies and others to cause persistent neurobehavioral effects. The current study extended the OP series tested to parathion. On postnatal days 1-4, we administered parathion to male and female Sprague-Dawley rat pups at doses below the threshold for overt signs of systemic toxicity and spanning the threshold for barely-detectable cholinesterase inhibition (0, 0.1 or 0.2 mg/kg/day). Significant and selective long-term behavioral effects were seen in adolescence and young adulthood. The higher dose (0.2 mg/kg/day) caused a significant ($p<0.05$) increase in tactile sensorimotor startle response, but we did not detect a significant alteration of auditory prepulse inhibition of the tactile startle response with parathion. In the elevated plus maze the same parathion dose significantly ($p<0.05$) increased time spent on the open arms and significantly ($p<0.05$) increased number of center crossings relative to vehicle-treated controls. Novelty suppressed feeding, preference for sweetened chocolate milk and locomotor activity and its habituation were not significantly affected. This study demonstrates the selective long-term behavioral alterations caused by exposure to the OP parathion at a low dose that does not produce clinical signs of toxicity. In combination with our earlier work on chlorpyrifos and diazinon, the current study with parathion shows that different OPs evoke disparate, persistent behavioral outcomes, supporting the contribution of mechanisms other than cholinesterase inhibition as underlying causes of developmental neurotoxicity. These differing actions may provide a key to identify OPs that might pose greater neurotoxic risk with widespread, low-level exposures.

2276 DIISOPROPYLPHOSPHOROFLUORIDATE (DFP) INDUCED DIFFERENTIAL mRNA EXPRESSION OF BCL2 IS ONE OF THE MOLECULAR SYMPTOMS OF ORGANOPHOSPHATE INDUCED OPIDN IN HENS.

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Diisopropyl phosphorofluoridate (DFP) produces organophosphorus-ester induced delayed neurotoxicity (OPIDN) in various sensitive species. We studied the effect of single dose of DFP (1.7 mg / kg /s.c.) on the mRNA expression of Bcl-2, which plays a major antiapoptotic role by counteracting the activation of apoptosis effectors such as caspases. The hens were sacrificed at different time points i.e, 1,2,5, 10 and 20 days. Total RNA was extracted from the following brain regions: cerebrum, cerebellum, and brainstem as well as spinal cord. Northern blots prepared using standard protocols were hybridized with Bcl2 probe as well as with beta-actin and 28S RNA cDNA (control) probes. The results indicate a differential / spatial / temporal regulation of Bcl2 mRNA levels. Cortex showed an induction of 144%, 169%, 143%, 160%, and 155% at 1,2,5,10 and 20 days respectively. Cerebellum showed an induction of 144%, 207%, 183%, 148%, at 1to 10 days respectively, while the levels came back to control levels at 20 days. Brainstem showed an inhibition to 51% at 1 day, followed by control levels for the rest of the time points. Spinal cord showed near control levels in most of the time points except at 10 days where its levels were 145%. Persistence induction in less susceptible regions (cortex and cerebellum) and inhibition or near control levels in the highly susceptible regions of the CNS (brainstem and spinal cord) indicate a complex injury-induced response triggered early on by the CNS during the DFP-induced OPIDN. Hence our results indicate differential expression of Bcl2 is either one of the factors involved in the development of OPIDN or the result of progressive changes taking place during OPIDN.

2277 TRANSFER OF NEUROTOXICANTS (MALATHION AND LEAD ACETATE) IN COMBINATION THROUGH *IN VITRO* BLOOD-BRAIN BARRIER SYSTEMS.

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Organophosphate (OP) and lead-induced toxicities remain a problem for humans and animals and combined exposures are a possibility. We previously noted that OP insecticides such as malathion and chlorpyrifos could disturb the integrity of in vitro blood-brain barrier (BBB) systems (Neurotoxicol 26,277; SOT 2007 Abstract 707). The present study examined the possibility that OP exposure could alter the transfer of lead through the BBB. Transport of malathion (10 uM), malaoxon (1 uM) and lead acetate (1 and 10 uM) were examined in systems using rat astrocytes co-cultured with rat endothelial (RBE4) cells or bovine endothelial cells on lower and upper sides, respectively, of 0.45 micron collagen-coated inserts in 6-well plates. The lead was applied above the insert 10 hr after malathion or malaoxon and 14 hr later passages of the OP compounds and lead through the BBB were determined below the insert by acetylcholinesterase (AChE) inhibition in underlying neuroblastoma cells or by atomic absorption, respectively. Results indicated that the dual treatment could decrease resistance of the BBB containing bovine cells by 7-24% over that noted following treatments with the OP compounds or lead alone. The ratio of lead under the insert compared to that applied to the BBB was higher for the rat endothelial cell-astrocyte co-culture than for the bovine endothelial cell-astrocyte co-culture (>30% and <10%, respectively). Effects of malaoxon on AChE inhibition in neuroblastoma cells under the BBB were greater than those of protoxicant malathion, suggesting limited capability for toxicant biotransformation in these BBB model systems. They do appear to have, however, potential for examining the influence of one neurotoxicant on the transport of another.

2278 ORGANOPHOSPHORUS PESTICIDES (OPS) DO NOT INTERACT DIRECTLY WITH AIRWAY NERVES TO CAUSE NEURONAL M2 RECEPTOR DYSFUNCTION.

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Epidemiological studies link OP exposures to asthma, and in support of this hypothesis we have shown that chlorpyrifos, diazinon and parathion cause airway hyperreactivity in guinea pigs at doses that do not inhibit acetylcholinesterase (AChE). This effect is apparent 24 hr after exposure and involves dysfunction of autoinhibitory M2 muscarinic receptors on parasympathetic nerves supplying the airways. Whether OPs interact directly with airway nerves to antagonize M2 function or downregulate M2 expression, as has been shown to occur in the brain, is not known. To test for direct antagonism, we determined whether acute OP administration would potentiate nerve-induced smooth muscle contractions. However, neither parathion nor paraoxon had any effect on vagally-induced bronchoconstriction in intact guinea pigs or isolated trachea when administered acutely at levels that did not inhibit AChE. A lack of direct antagonism was further suggested by ligand binding studies using 3H-NMS or 3H-QNB to label membranes isolated from the hearts of guinea pigs exhibiting OP-induced airway hyperreactivity. Quantitative RT-PCR analyses of M2 mRNA in human neuroblastoma cells or primary guinea pig parasympathetic nerve cultures indicated that a 4 hr exposure to varying concentrations of paraoxon had no effect on M2 transcript expression. 3H-NMS-labeling of surface M2 in COS cells transfected with cDNA encoding full-length human M2 or primary sympathetic neuronal cell cultures showed that neither parathion nor paraoxon altered M2 receptor internalization in the absence or presence of carbachol. In summary, this study suggests that neither direct antagonism nor downregulated expression of M2 receptors contributes to OP effects on M2 receptors in parasympathetic neurons, adding to the growing evidence that not all OP neurotoxicity can be explained by cholinergic mechanisms. These studies were supported by NIEHS (grants ES014521 and ES014601).

2279 SPECTRAL EVALUATION OF ELECTROGRAPHIC SEIZURES AFTER EXPOSURE TO ORGANOPHOSPHORUS COMPOUNDS.

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The potential for exposure to organophosphorus (OP) nerve agents exists on the battlefield and even in urban areas due to the current terrorist's threats. Control of seizures induced by OP and means to mitigate central nervous system damage continue to be major military and civilian research goals. Soman exposure induces status epilepticus (SE), through overexcitation of cholinergic pathways followed by excitatory amino-acid-induced brain injury. N-Acetyl-β-aspartylglutamate

(β-NAAG) already showed efficacy against hypoxia- and NMDA-induced injury in vitro and ischemic spinal cord injury in vivo. Male rats were pretreated with the oxime HI-6 (125 mg/kg, i.p.) 30 min before the injection of soman (110 μg/kg, s.c.). One min after soman exposure, rats were injected with atropine SO4 (2 mg/kg, i.m.). At 30 min post soman exposure, diazepam (DZP, 10 mg/kg, s.c.) was injected for treatment of SE. Following the injection of DZP, a group of animals received β-NAAG (4 and 8 μmols, i.c.v.) or its vehicle. EEG was recorded 24 hours/day for fifteen days. At this time, all exposed animals were euthanized and their brains removed and processed for silver staining. Through the use of a customized Matlab program (Mathworks, Vers. 7), alterations in the EEG power spectra were evaluated. Magnitude of the power spectra on delta band remained increased in rats that had SE. This alteration persisted during all the entire observation period. β-NAAG-treated rats (8 μmols) had a reduction in the power spectra on delta band and also in electrographic seizure activity during the 1st day after exposure. However, these rats still showed marked brain damage. Continuous seizure activity persisted even after the injection of DZP + neuroprotectant and appears to be the main cause of brain damage. Therefore, repeated treatment with anticonvulsants/neuroprotectants may be needed to reduce or avoid OP neuroexcitotoxicity.

2280 THE RELATION BETWEEN SPONGY CHANGE OF SPINAL CORD AND EXPRESSION OF 2', 3'-CYCLIC NUCLEOTIDE 3'-PHOSPHODIESTERASE (CNP) INDUCED BY ANILINE IN RATS.

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A spongy change in the spinal cord white matter was observed in 4-week-old rats given aniline (Okazaki et al. J. Vet. Med. Sci. 63: 539, 2001. Okazaki et al. J. Tox. Pathol. 14: 19, 2001). Although this change consisted of a splitting of the myelin sheath at the ultrastructural level, the mechanism is unknown. This study was conducted to identify the mechanism of spongy change in aniline-treated rats.
<Materials and Methods>
Thirty-five 4-week-old male, Crl:CD(SD) rats were orally treated once with 1000 mg/kg aniline (Day 1). The rats were chronologically (Day 2, 3, 4, 5, 8, 10, 12) killed by exsanguination from the right atrium and perfused via the left ventricle with 4% paraformaldehyde. The spinal cords were removed, embedded in paraffin, sectioned at 5 microns, and stained with HE, followed by immunostaining. Furthermore, the thoracic spinal cords were examined by electron microscopy.
<Results>
The spongy change in the spinal cord white matter was first detected on Day 5 in the histopathologic examination. The incidence and severity of the lesions, especially in the lateral and ventral funiculus in the thoracic spinal cord white matter, increased prominently on Day 8 and 10. The immunohistochemical staining with anti-CNPase revealed positive reactions along the cytoplasm of the oligodendroglia in all rats. However, granular products that reacted only with anti-CNPase were consistently seen in the swollen cytoplasm of the oligodendroglia of the aniline-treated rats on Day 4. In the electron microscopic examination, free ribosomes and rough endoplasmic reticulums in the cytoplasm of the oligodendroglia increased on Day 3 and 4.
<Discussion>
These data suggest that the decrease in CNPase expression may contribute to the changes in the myelin morphology observed in aniline-intoxication. This is the first description of CNPase expression related to the spongy change of the spinal cord white matter in aniline-treated rats.

2281 CYP1A2*1F AND PON1 Q192R GENETIC POLYMORPHISMS AS SUSCEPTIBILITY DETERMINANTS FOR NEUROLOGICAL EFFECTS IN MEXICAN FARMERS EXPOSED TO ORGANOPHOSPHOROUS PESTICIDES.

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Most organophosphate pesticides (OP) require bioactivation via the CYP450 family to form the toxic oxons, which exerts the neurotoxic effects and are deactivated by paraoxonase (PON1) enzyme. CYP1A2*1F polymorphism is related to higher activity and may be a risk factor to develop OP toxicity, whereas PON1 Q192R polymorphism that is substrate-dependent, has been suggested to participate in OP susceptibility. Thus, the aim of this study was to determine the role of CYP1A2*1F and PON1 Q192R polymorphisms on neurological damage in agricultural workers exposed to a mixture of pesticides (including OP) in Yucatan State, Mexico. A cross-sectional study was conducted in 80 subjects with a strong Mayan ascendancy. A structured questionnaire was applied to evaluate lifestyle, health status, pesticide exposure and symptoms of neurological damage. A chronic OP exposure index was created and CYP1A2*1F and PON1 Q192R polymorphisms were determined by Real Time-PCR. The allelic frequency for CYP1A2*1F was 0.82 and 0.52 for PON1 192R. Participants (mean age of 43 years) had 1-54 years working in agriculture, and 69% reported adverse neurological symptoms after applying pesticides. Homozygote *1F/*1F subjects showed an increased risk for presenting more than 10 symptoms, while homozygotes for the 192R or *1F alleles showed a higher risk for presenting an intoxication episode. No interaction between both polymorphisms was observed, probably due to the complex pesticides mixture or the small number of individuals with each haplotype combination. These data suggest that carrying the *1F/*1F or 192RR genotypes confer a tendency of increased risks of neurological damage, and they may individually participate in OP neurotoxicity. (Project supported by CONACYT, Grant CO-134).

2282 DETERMINATION OF LC50S FOR ROUNDUP AND MANZATE IN CAENORHABDITIS ELEGANS.

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Previous studies have demonstrated a positive correlation between high levels of pesticide and Parkinson's Disease (PD). Indeed, PD is also known to be generally more prevalent in agricultural communities where agrochemical usage is high. In order to further explore this potential relationship, we conducted a series of experiments to lay the groundwork for future testing of the following hypothesis: Two common pesticides, RoundUp (glyphosate) and Manzate (manganese/zinc ethylene-bis-dithiocarbamate) may lead to neuronal degeneration by mitochondrial inhibition. As a first step, we acutely exposed wild type (N22) Caenorhabditis elegans to varying concentrations of RoundUp or Manzate for 30 min. To further model environmental exposure, a third population of worms was exposed first to RoundUp for 30 min, followed by a 30-min incubation with Manzate. The LC50 was determined by counting the number of live worms remaining 24 hours post-exposure. The LC50 for RoundUp was found to be 4.9% (95% confidence interval: 4.6-5.2%; r2 = 0.8593 for curve), for Manzate, ~0.80% (95% confidence interval: 0.66-0.86%; r2 = 0.9719 for curve), and for the combination treatment was ~12.5% (95% confidence interval: 12.3-13.3%; r2 = 0.6670). The values for the individual treatments correlated well with the recommended usage concentrations for each agrochemical (2% for RU and 0.658% MZ). Additional experiments were conducted with RoundUp alone to determine if exposure of adult worms affected the viability of resulting progeny. Fifty washed adult worms from the dose-response experiments were moved to new agar plates 30 h post-treatment and allowed to grow up overnight. Worms were counted the next day for presence of progeny. These data suggested that progeny viability is not affected by adult treatment. Taken together our results indicate that our model system using C. elegans is appropriate and useful for further studies investigating potential mechanisms involved in neuronal degeneration related to pesticide exposure.

2283 EFFECTS OF ATRAZINE AND ITS METABOLITES ON THE UPTAKE OF DOPAMINE INTO RAT STRIATAL SYNAPTIC VESICLES AND SYNAPTOSOMES.

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Studies have shown that both *in vivo* and *in vitro* exposure to the herbicide atrazine (ATR) results in dopaminergic neurotoxicity manifested by decreased striatal dopamine (DA) levels. However, the mechanism behind this reduction is not well known. Decreased DA level could be caused by effects of ATR on vesicular and/or synaptosomal uptake resulting in disruption of vesicular DA storage and/or cellular uptake. Hence, we investigated the effects of in vitro ATR exposure on DA uptake into rat striatal synaptosomes and vesicles. In addition to ATR, effects of its major metabolites, didealkyl atrazine (DACT), desethyl atrazine (DE) and desiopropyl atrazine (DIP) were investigated. ATR inhibited DA uptake into synaptic vesicles in a dose dependent manner and the maximum inhibition being with 250 μM to about 50% of control. Of the three ATR metabolites tested, DACT did not affect the vesicular DA uptake whereas DE and DIP significantly decreased vesicular DA uptake with the effect of 100 μM being similar to the effect of the same concentration of ATR. Kinetic analysis of DA uptake showed that ATR significantly decreased vesicular uptake V_{max} while K_m value was not affected. The degree of inhibition decreased when concentration of cold DA was increased. Contrary to the inhibitory effects on vesicular DA uptake, synaptosomal DA uptake was marginally (5-10%) increased by ATR, DIP and DE, but not by DACT, at concentrations of 100 μM or less. As a result, ATR, DIP and DE increased the synaptosomal/vesicular (DAT/VMAT-2) uptake ratio. Collectively, results from this study suggest that ATR and two of its metabolites, DIP and DE, but not its major metabolite DACT, decrease striatal DA levels, at least in part, by increasing cytosolic DA, thereby promoting its breakdown. Partial support provided by: P20 RR017661 (NIH).

2284 LIFESPAN ANALYSIS OF DIAMINOCHLOROTRIAZINE (DACT) MODIFIED PROTEINS FOLLOWING ATRAZINE EXPOSURE IN RATS.

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Atrazine is a commonly used herbicide and groundwater contaminant, which has been shown to disrupt luteinizing hormone release from the pituitary. Atrazine is quickly metabolized to the electrophilic diaminochlorotriazine (DACT) following oral exposure, which can react with nucelophilic amino acids. DACT has been shown to form covalent adducts with numerous proteins in the pituitary, but lifespan of these modified proteins is unknown. In this study, we looked at relative abundance of immunodetected DACT modified pituitary proteins, which were separated by 2D electrophoresis. Eight female wistar rats were given 200 mg/kg atrazine or carboxymethylcellulose (control) for 4 days via oral gavages. Four of the animals given atrazine were sacrificed following the final dose, while the other four were sacrificed after a 4 day withdraw period. 2D gel electrophoresis of pituitary lysates and immunodetection of DACT adducts indicated that on day 8, 97/221 spots detected on day 4 were absent, 40/221 were reduced by 20% or more, 54/221 showed less than 20% difference, 26/221 were increased by 20% or more on day 8. In addition 4 spots were present on day 8 that were not detected on day 4. This study gives us the first insight into the lifespan of DACT modified proteins following atrazine exposure.

2285 PREVENTION BY ANTIOXIDANTS OR NMDA RECEPTOR ANTAGONIST OF CARBOFURAN-INDUCED ALTERATIONS IN BIOMARKERS OF NEURODEGENERATION AND NEUROINFLAMMATION IN RAT BRAIN.

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Carbamate insecticides produce their toxicity in brain primarily by acetylcholinesterase (AChE) suppression. However, mechanisms involved in neuronal damage are linked to excess free radicals generation. Evaluation of selected brain regions (cortex, amygdala, and hippocampus) that are involved in initiation and propagation of seizures, by a single acute dose of carbofuran (1.5 mg/kg, sc), revealed significant ($P<0.05$) increases in F2-IsoPs and F4-NeuroPs (ROS markers), nitric oxide (NO, RNS marker), and PGE2 (neuroinflammation marker), and significant declines in high-energy phosphates (ATP and PCr) and their metabolites. A time course study indicated a 2-4-fold increase in NO prior to onset of clinical signs. In addition, significant declines were observed in dendritic lengths and spine density in pyramidal neurons of hippocampal CA1 sector. Maximal changes in these parameters occurred during 30-60 min, which is the time of peak cholinergic toxicity. Pretreatment of rats with an antioxidant (PBN, 200 mg/kg, ip; or vit E, 100 mg/kg, ip/day for 3 days) or NMDA receptor antagonist memantine (18 mg/kg, sc) provided significant protection against carbofuran induced changes in F2-IsoPs, F4-NeuroPs, NO, PGE2, ATP and PCr and their metabolites. Memantine also prevented alterations in dendritic system. Thus, in addition to AChE inhibition, carbofuran-induced changes in biomarkers of neurodegeneration and neuroinflammation can be prevented by an antioxidant or NMDA receptor antagonist.

2286 PHYSICO-CHEMICAL CHARACTERIZATION OF STEALTH LIPOSOMES ENCAPSULATING A HYDRLYZING ENZYME EMPLOYED IN ORGANOPHOSPHORUS ANTAGONISM.

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The present work focuses on the preparation and characterization of sterically stabilized liposomes (SL*) containing the recombinant organophosphorus hydrolysing enzyme, phosphotriesterase (OPH). The liposomal-OPH carrier system has previously been shown to provide an enhanced protective effect in the antagonism of organophosphorus intoxication, presenting a more effective and longer acting therapy with less toxicity than the most commonly used antidotes. Physicochemical characterization of the liposomal OPH delivery system has provided information relevant to the in vitro stability and in vivo fate, including osmolarity, pH, viscosity, encapsulation efficiency of the SL* preparation and the surface potential of the liposomes. The membrane rigidity and the impact of OPH enzyme on the membrane was studied by electron-paramagnetic resonance (EPR) spectroscopy using spin probes. The in vitro stability of the preparation, the vesicle size distribution and its alteration during 3-week storage were followed by dynamic light scattering measurements. Our results can serve as a model for designing other intravenously administered enzyme encapsulating nano-delivery systems for the treatment of intoxications.

These studies were supported in part by research funds from the National Institute of Health (Grant #: 5 U01 NS058035-02).

2287 DECREASE OF DOPAMINE LEVELS AFTER GLYPHOSATE HERBICIDE TREATMENT IN RATS.

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Glyphosate (N-phosphonometil-glicine) is a synthetic amino acid with an empiric formula of C3H8NO5P. It is an organophosphate herbicide with no anticholinesterase activity. Accidental ingestion of glyphosate is generally associated with mild to moderate effects such as spasms, panting, shaking, hind-leg ataxia and muscle spasms. Because of toxicological data are needed to further define the use safety of this herbicide, the objective of the present study was to investigate neurochemical effects following the administration of glyphosate (75, 150 and 800 mg/kg/day, orally for 5 days) in male Wistar rats (n=6/group). Animals were sacrificed 24 hours following glyphosate administration and their brains were rapidly removed. The frontal cortex, hippocampus, hypothalamus, midbrain and striatum were dissected and analyzed for content of dopamine (DA) and its metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) using a HPLC method with electrochemical detection. A dopamine depleting, dose-dependent, effect was produced by this herbicide. Glyphosate, at the lowest dose, only decreased DA levels in hippocampus (20% $P<0.05$) and hypothalamus (18% $P<0.001$), and increased DOPAC and HVA levels in striatum (15% $P<0.01$). Glyphosate, at the highest dose, decreased DA levels in frontal cortex (53% $P<0.01$), hippocampus (40% $P<0.001$), hypothalamus (25% $P<0.001$), striatum (22% $P<0.001$) and midbrain (15% $P<0.001$) respect to corn oil controls. Also, at the two highest doses, glyphosate caused a statistically significant increase in the metabolite DOPAC and HVA levels. These results may prove a valuable contribution for the planning of toxicological investigations and risk assessment of this herbicide. This work has been supported by projects No. AGL2005-05320-C02-02 and Consolider-Ingenio 2010 No. CSD2007-063 (MEC), Spain.

2288 BRAIN CHOLINESTERASE INHIBITION AND DEPRESSION OF THE PHOTIC AFTER DISCHARGE (PHAD) OF FLASH EVOKED POTENTIALS (FEPS) IN LONG EVANS RATS FOLLOWING ACUTE OR REPEATED EXPOSURES TO A MIXTURE OF CARBARYL AND PROPOXUR.

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Carbaryl and propoxur are *N*-methyl carbamate pesticides (NMCs) which are part of the EPA's cumulative risk assessments for NMCs. These NMCs inhibit cholinesterase (ChE) activity and may lead to cholinergic disruption of CNS function. We used decreases in the PhAD of FEPs to indicate altered CNS function. FEPs were recorded in male Long Evans rats (>60 days) following acute treatment with 0, 3, 10, 45 or 75 mg/kg or repeated administration (14 days) of 0, 3, 10, 30 or 45 mg/kg of the mixture (ratio propoxur:carbaryl = 1:1.45). One week before testing, rats were implanted with epidural electrodes. FEPs were recorded for 2 days to develop the PhAD. On the 3rd day, pupils were dilated (40 min) and rats dosed (30 min) before testing. Immediately after testing, rats were sacrificed, the brains removed, and stored at -80°C until determination of ChE levels by radiometric assay. Both acute and repeated treatments inhibited brain ChE activity to a similar extent. Three-45 mg/kg of the mixtures inhibited 14.7-56.4% (acute) and 11.3-53.5% (repeated) of brain ChE. Acute treatment with 75 mg/kg of the mixture resulted in 63.4% inhibition of ChE. The PhAD was significantly decreased after repeated exposures, and nearly significantly after acute treatment. Brain ChE levels significantly correlated with PhAD duration after both exposures. The early portion of the FEPs (peak N_{36}) was not altered with either exposure duration. The results indicate that acute and repeated exposures to mixture of NMCs produced similar levels of brain ChE inhibition, and similar relationships between brain ChE levels and PhAD duration. They also support the conclusion that inhibition of brain ChE by NMCs disrupts the cortical processing related to the PhAD. [1]*Supported by a NRC Fellowship. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.*

2289 ALDEHYDE DEHYDROGENASE INHIBITION BY METABOLITES OF MOLINATE, A THIOCARBAMATE HERBICIDE.

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Molinate is a thiocarbamate herbicide, commonly used as a pre-emergent in rice patty fields, which has been shown to cause neurotoxicity. Molinate undergoes oxidation to molinate sulfoxide and is further oxidized to molinate sulfone, an electrophile reactive toward thiols. Previous work demonstrated a decrease in aldehyde dehydrogenase (ALDH) activity in rats treated with molinate. These findings suggest the sulfone metabolite of molinate, and perhaps the sulfoxide metabolite, to be an inhibitor of ALDH, potentially modifying the active-site cysteine. ALDH is an enzyme important in the catabolism of many neurotransmitters, and inhibition of these catabolic processes may lead to the accumulation of neurotoxic metabolites such as 3,4-dihydroxyphenylacetaldehyde (DOPAL). The hypothesis of this study is molinate and both metabolites inhibit ALDH, and molinate sulfone is the most potent inhibitor. To test this hypothesis, molinate sulfoxide and molinate sulfone were synthesized, and various model systems were used to measure ALDH activity, including rat brain ALDH, rat striatal mitochondria, synaptosomes, and human recombinant ALDH (hrALDH). The conversion of NAD^+ to NADH was monitored using UV spectrophotometry for hrALDH. Rat brain ALDH, synaptosome, and mitochondria assays utilized HPLC to monitor the concentrations of DOPAL and DOPAC over time. Using hrALDH with the substrate propionaldehyde, the relative potency of each metabolite could be determined. Molinate sulfone was found to be the most potent inhibitor of ALDH, followed by molinate, and then molinate sulfoxide. These results demonstrate molinate and both metabolites inhibit ALDH activity, and molinate sulfone causes an increase of the endogenous neurotoxin, DOPAL.

2290 THE POSSIBLE ROLE OF CHLOROACETALDEHYDE IN 2-CHLOROETHANOL NEUROTOXICITY.

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2-Chloroethanol is personally used on grapevines to hasten sprouting by farmers in Taiwan. However, accident intoxication of farmers had been reported annually, and indicated it to be up to 40 % of mortality of cases. In previous studies, we found that there were no gross pathological findings in animal organs of 2-chloroethanol intoxication. We also found chloroacetaldehyde, a major metabolite of 2-chloroethanol, was present high concentrations in rat's blood. In order to clarify the toxicity of 2-chloroethanol, we performed the acute toxicity test of 2-chloroethanol in rats. Furthermore, 2-chloroethanol and chloroacetaldehyde were individually added into the organ bath with isolated mouse phrenic nerve diaphragm. Both nerve- and muscle-evoked twitches were recorded. We found that chloroacetaldehyde caused contracture in stimulated or quiescent isolated mouse phrenic nerve diaphragm evoke, but not of 2-chloroethanol. These results indicated that chloroacetaldehyde might play a major role in the neurotoxicity of 2-chlorothanol.

2291 ESTERASE PROFILES AND QSAR FOR A SERIES OF DIALKYL-O-(2-HYDROPERFLUOROPROP-2-YL) PHOSPHATES (PFP).

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Esterase profiles were obtained for a series of PFP (R = Me, Et, Pr, i-Pr, n-Bu, i-Bu, n-Pent, i-Pent, and n-Hex) by determining the bimolecular rate constants of inhibition (ki) against acetylcholinesterase (AChE), neuropathy target esterase (NTE), butyrylcholinesterase (BChE), and carboxylesterase (CaE). Log ki was 1.04 to 5.65 for NTE and 2.34 to 4.89 for AChE. PFP with short R-groups (Me, Et) were more potent inhibitors of AChE than NTE. Anti-NTE activity and NTE selectivity [ki(NTE)/ki(AChE)] increased with rising hydrophobicity. High NTE selectivity (2.45 to 7.45) for R ≥ Pr suggests that these compounds would be neuropathic at sublethal doses. Log ki was 2.52 to 6.56 for BChE and 3.98 to 7.21 for CaE. Potency against both esterases increased with rising hydrophobicity. QSAR analysis found a parabolic dependence of NTE selectivity on R hydrophobicity. To consider the potential role of nonspecific esterases in PFP toxicity, relationships between PFP structure and ki(CaE)/ki(AChE), ki(BChE)/ki(AChE), ki(CaE)/ki(NTE), and ki(BChE)/ki(NTE) were analyzed by polynomial regression. A family of parabolic equations was obtained, which along with the equation for ki(NTE)/ki(AChE), describe the change in esterase profile of PFP with hydrophobicity. The data suggest that interactions with "nonspecific" esterases (BChE and CaE) would have different potential effects on acute and delayed neurotoxicity by affecting binding to AChE and NTE, respectively. Overall, however, the data agree with previous results showing low neuropathic potential of OP compounds with short R (Me, Et) and high neuropathic potential for long-R compounds (Supported by ISTC project #3130).

2292 STRUCTURAL INSIGHTS INTO THE HUMAN NEUROPATHY TARGET ESTERASE (NTE) CATALYTIC DOMAIN (NEST).

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NTE is a transmembrane protein of unknown structure and function whose inhibition and aging produces axonopathy. To test the hypothesis that aging results in a conformational change distinct from that seen with inhibition, we modeled NEST using the crystal structure of human cytosolic phospholipase A2 (cPLA2; PDB ID 1CJY) as a template. The INUB server was used to obtain sequence alignments and secondary structure predictions for NEST against cPLA2. We used O and PyMol to build the NEST model, followed by refinement via simulated annealing using the Crystallography and NMR system. We used near- and far-UV circular dichroism spectroscopy (CD) to study secondary structure and tertiary structural changes associated with inhibition and aging of NEST. Molecular modeling showed that NEST has a modified α/β hydrolase fold with 16 α-helices and 12 β-strands. The catalytic Ser966 was located in a characteristic nucleophilic elbow. We confirmed our earlier findings that the NTE active site consists of a Ser966-Asp1086 catalytic dyad instead of a Ser966-Asp1086-Asp960 triad. Far-UV CD revealed that NEST contains 38% α-helices and 28% β-strands, giving a helix:strand ratio of 1.36, in close agreement to the ratio of 1.33 seen in our model. The conformational change predicted from modeling inhibition of NEST by the nonaging inhibitor phenylmethanesulfonylfluoride (PMSF) (root-mean square deviation, RMSD = 1.6 Å) was similar to that from modeling inhibition/aging by the aging inhibitor diisopropylphosphorofluoridate (DFP) (RMSD = 1.4 Å). Near-UV CD results were consistent with modeling results: spectra of NEST treated with 1mM PMSF or DFP were virtually superimposable. Thus, modeling and CD results are in agreement that inhibition and inhibition/aging of NEST result in similar degrees of conformational change, leaving open the question of why aging is necessary for the initiation of OP-induced axonopathy (Funded by ARO DAAD19-02-1-0388, MEDC, and NIH ES07062).

2293 A REVIEW OF NONCLINICAL DEVELOPMENT STRATEGIES USED TO SUPPORT PHASE I CLINICAL TRIALS WITH INHALED PHARMACEUTICALS.

A. Gibbs and P. Smith. *Programme Management, Covance, Harrogate, United Kingdom.* Sponsor: C. Springall.

The preclinical development strategy used prior to first-in-man Phase I clinical studies with inhaled pharmaceuticals is a complex area. The inhalation route of administration is used for pharmaceuticals intended both for the local treatment respiratory disease, as well as for systemic delivery aimed at the treatment of a range of indications. Thus, the nonclinical approach to development largely depends on the predicted extent of systemic absorption in the human, compared to that observed in the animal species. This will direct the balance between evaluation of local and systemic toxicity as well as the design of the other aspects of the nonclinical development programme, such as safety pharmacology. The ever increasing pressures faced by drug companies at the early stages of drug development mean that getting a compound into the clinic for the first time as quickly as possible (or quickly identifying those compounds that are not suitable for early clinical development) is more important than ever. Thus, when designing inhalation development programmes the early evaluation of pharmaco/toxicokinetic parameters is essential to allow critical decisions regarding the pivotal studies to be made. Other important considerations include, choice and number of species, dose level selection (MTD, or maximum feasible, or multiple of clinical), dose route selection (inhalation alone, systemic), requirement for safety pharmacology, genetic toxicology and ADME. Inhalation development can also be subsequent to development by an oral or parenteral dose route in which case the development strategy will be abbreviated due to the existing systemic toxicity data package. Other factors impacting on the nonclinical development of respiratory pharmaceuticals include formulation issues, dosimetry as well as considerations relating to the practical aspects of generating a suitable aerosol of the drug for administration in GLP toxicology studies. A review of current best practices in the early nonclinical development of inhaled drugs will be presented and conclusions drawn relating to the approaches adopted.

2294 DRUG-INDUCED TOXICITY AND ITS REVERSIBILITY IN THE REGULATORY NON-CLINICAL STUDIES WITH NEW DRUGS IN JAPAN.

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Drug-induced toxicity with therapeutic class and its reversibility were surveyed in more than 150 new drug applications for latest 8 years in Japan. The findings of toxicity consisting of various parameters were examined in repeated-dose toxicity studies, and functional toxicity and reversibility in generative organs were observed in reproduction studies. Further, toxicity profiles between adult and juvenile animals were comparatively analyzed. Most of toxicity findings were well recovered after one- to three-month withdrawal period in rodent and non-rodent species. Irreversible and delayed reversibility findings were recognized in histopathological examination. Liver and kidneys were major targets, although gastrointestinal tract, heart, eye, brain, bone, muscle and reproductive organs were involved. The changes included swelling, degeneration, atrophy, metaplasia, necrosis, fibrosis, calcification, proliferation and others. There was a tendency to delayed reversibility of findings due to the hormonal effects, immunological responses and/or multi-organ lesions. In juvenile animals, the dosage level rather than the targeted profile was a prominent factor in comparison with adult animals.The reversible findings in targeted organs were characterized in the approved drugs.

2295 OPTIMISATION OF PLETHYSMOGRAPHY CONDITIONS FOR USE IN REPEAT DOSE TOXICITY STUDIES.

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Barometric whole body plethysmography (WBP) is a non-invasive technique for assessing ventilation. To stabilise the respiratory signal rats, are typically allowed 60 min to settle prior to recording. However, to permit inclusion of WBP into repeat dose studies, conditions that enable rats to settle more quickly (defined as a stable respiratory signal) need to be identified. The objective of this study was to derive these optimal conditions using primarily the respiratory rate measurement. Male Han Wistar rats (weight range 342 to 399g at start of study) were used in a factorial design format. Factors assessed included length of settling time, lighting conditions in the plethysmography room and availability of shade within the plethysmograph (n=4 for each factor). Habituation was also assessed as a consequence of repeated measurements within the same rats. On test days, rats were placed in a plethysmograph (with or without shade) and were allowed 5 or 15 min to settle in either light or dark conditions. Respiratory signals were then recorded for a further 5 min. Rats were tested 8 times over a 26 day period. Respiratory rates were lower in rats that were allowed 15 rather than 5 min to settle (respectively, day –2, 190±22 bpm and 237±16 bpm ($p<0.1$); day 15, 202±11 bpm and 145±10 ($p<0.001$); day 24, 133±14 bpm and 199±16 bpm ($p<0.005$)). Neither the lighting conditions nor the availability of shade had a significant effect on the stabilisation of respiration. Rats also habitutated to the plethysmograph with repeated exposure to the plethysmograph study as evident by lower respiratory rates on the last test day. In conclusion, the length of settling time within and habituation to the plethysmograph were the most influential factors. Overall, respiratory rates are higher than those in safety pharmacology studies so to incorporate WBP into repeat dose studies, it would be desirable to habituate animals to the plethysmograph during a pre-study period and have a longer settling time. This would be dependent upon study size but could be achieved by a staggered start.

2296 DETERMINATION OF TIDAL VOLUME AND RESPIRATORY RATE BY PNEUMOTACHOGRAPHY IN THE MARMOSET.

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Evaluation of respiratory parameters is an essential feature of safety testing. An effective way to evaluate the possibility that a test item might influence respiration is the determination of tidal volume and respiratory rate. The traditional method for measurement of tidal volume in rodents relies on pressure changes in a whole body plethysmograph. Since the marmoset (Callithrix jacchus, 1) is not commonly used in safety pharmacology studies, an alternative method for determination of tidal volume in conjunction with respiratory rate was evaluated. Tidal volume and respiration rate of 16 animals under sedation were determined using a pneumotachograph. Ten minutes after induction of anaesthesia (50 mg/kg ketamine, 0.5 mg/kg diazepam) animals were intubated using a laryngoscope and a self-made tracheal tube (diameter inside: 1,5 mm, diameter outside: 2,0 mm) and connected to Validyne Pressure Transducer (Ponemah) aligned with a Carrier Signal Conditioner 13-7715-35 (CSC). The Ponemah Physiology Platform Model P3 Plus, Version 4.10-SP1 and software module Pulmonary airflow: PNM-PAF100W were used for analysis. Airflow was monitored continuously based upon differential pressure measurements. Once a regular breathing pattern after intubation was seen, breath per minute (BPM) and tidal volume VT (ml/breath) data were accepted for further analysis. Five minute interval analysis revealed an average of 50 BPM (SD: ± 20) and a mean tidal volume (VT) of 0.09 mL (SD: ± 0.057). In a former study manual counting in non-sedated animals revealed 96 to 101 BPM (own data). In unrestrained animals, 36-44 BPM were registered by telemetry (2). In summary, this investigation demonstrated the feasibility of respiratory rate and tidal volume measurements in sedated, intubated marmosets. (1) Mansfield (2003) Marmoset models commonly used in biomedical research. Comp Med 53, 383-392 (2) Horii (2002) Development of telemetry system in the common marmoset—cardiovascular effects of astemizole and nicardipine. J Toxicol Sci 27, 123-130

2297 PULMONARY DELIVERY OF MEASLES VACCINE: NON-HUMAN PRIMATE SAFETY STUDY.

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The pulmonary delivery of measles vaccine is of great interest for vaccination programs in developing countries, since it would bring benefits in terms of needle-free delivery and economical approaches to vaccination campaigns. Measles results in significant child mortality in developing countries every year. Since the pulmonary route is the natural route of infection by measles, pulmonary delivery is expected to be an effective route of immunisation. In the study reported here, we have evaluated the safety of pulmonary delivery of Edmonston Zagreb measles vaccine in cynomolgus monkey, a non-human primate which is susceptible to infection with measles. Vaccine preparations were administered to groups of monkeys on two occasions (Day 1 and Day 21) by nebuliser, at 5 fold the intended clinical dose (in terms of viral particles). Separate treatment groups received administrations with low droplet size (MMAD 1-5 μM), high droplet size (10 μM) measles vaccine, or placebo solution. In life clinical evaluations and laboratory investigations were performed and animals were sacrificed on Day 8 and Day 43 for terminal and post-mortem investigations. Administrations were well tolerated without evidence of local effects in the eyes, nose, mouth and throat. There was no mortality during the study. No effects were seen on body weight evolution, food consumption, ophthalmology, rectal temperature or clinical pathology. Water consumption was increased in measles vaccine treated animals with a corresponding increase in urine volume. Immunogenicity and viremia following treatment were characterised. A full range of tissues was evaluated for histopathological changes and no treatment-related findings were observed. It was concluded that pulmonary administration of Edmonston Zagreb measles vaccine was well tolerated locally and did not result in any evidence of systemic toxicity.

2298 LONG TERM CONTINUOUS INTRAVENOUS INFUSION IN THE ALBINO RAT, BEAGLE DOG, CYNOMOLGUS MONKEY AND GÖTTINGEN MINI-PIG.

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Continuous intravenous infusion via an indwelling catheter is frequently used in preclinical safety evaluation studies to mimic the proposed clinical route or to achieve higher blood drug levels. These studies have been conducted at our laboratory for over 20 years. Control data was evaluated to determine any effects of continuous intravenous infusion on the body weight gain, food intake/appetence and clinical pathology parameters over a 6 to 9 month period. A medical grade silicone-based catheter was surgically implanted under general anesthesia in the vena cava via a femoral vein. Animals were continuously infused with saline at rates of 0.4 mL/h (rodents) or 4 mL/h (non-rodents) for 24 hours/day, for 6 to 9 months, using a jacket and tether system. Body weight gains and food intake were unaffected when comparing data with historical control values for non-catheterized animals. Small differences in the clinical pathology data were observed between the catheterized and non-catheterized animals that could be associated with the presence of a foreign material.

It is concluded that in our laboratory the presence of an indwelling catheter and continuous intravenous infusion of saline at a rate of 0.4 mL/h in albino rats or 4 mL/h in beagle dogs, cynomolgus monkey or Göttingen mini-pigs for 6 to 9 months had no adverse effects on growth compared with non-catheterized animals. Clinical pathology changes were minor and consistent with the presence of a foreign material. The infection rate for the longer term studies also remained compa-

rable to the incidence observed in our shorter term studies, of <1% in all these species. Our methods are considered appropriate for safety assessments of compounds intended for chronic administration via this route for continuous or intermittent infusion regimens.

2299 A METHOD FOR CONTINUOUS INTRAVENOUS INFUSION IN GOTTINGEN MINI-PIGS.

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The mini-pig has been shown to be a good pharmacological model due to several physiological and morphological similarities to humans. Issues including species sensitivity and ethical concerns have resulted in its increasing use as an alternative non-rodent species in toxicology studies. In response to the need for a mini-pig model of continuous intravenous infusion, our laboratory developed such a model using Gottingen minipigs. The Gottingen strain was selected due to its small size, sociability and ease of handling compared to other strains. As a validation, 3 males and 3 females were surgically cannulated 2 weeks before starting a "mock" treatment. A sterile polyurethane catheter was inserted into the femoral vein and advanced into the vena cava. Using a radio-opaque contrast media and fluoroscopy, the required catheter length and tip position were confirmed, ensuring consistent postion at approximately the level of the kidneys. Following implant and continuing until "mock" treatment, catheter patency was maintained by slow infusion of saline via a jacket and tether system, such that the infusate and pump were located outside the cage, leaving the pigs unencumbered. During the 14-day "mock" treatment, the rate of saline infusion was increased to 3 mL/kg/hour, to mimic a typical test article infusion rate. Parameters monitored during the study included mortality, clinical signs, body weight, food consumption, ECG, and clinical pathology. At termination, a macroscopic examination was performed; selected organs were weighed to provide background data; and infusion sites (with catheter tip) were examined histopathologically. Results demonstrated that the surgical and infusion procedures were appropriate and that the Gottingen mini-pig could be used in toxicology studies requiring continuous intravenous infusion. Subsequent to the validation, a 28-day continuous intravenous infusion study in Gottingen minipigs was successfully conducted, in which 8 control animals received a dextrose-based vehicle formulation, using similar methodology and parameters to those described above.

2300 NEW INITIATIVES TO ENSURE AVOIDANCE OF CROSS-CONTAMINATION IN THE VIVARIUM.

S. Doughty. *Covance Laboratories Ltd., Harrogate, United Kingdom.* Sponsor: D. Everett.

Over the last 10 years one of the most significant changes in regulatory toxicology has been the ever-increasing sensitivity of assay techniques to detect and measure test article in blood and other body fluids. This evolution has been highly valuable as medicines becomes ever more potent and exert their effect at lower plasma levels, which require more sensitive methods to allow accurate measurement.

There is no doubt that the ability to detect materials at very low levels has increased awareness of the presence of test article in control samples from toxicity studies. It may well be the case that some unwanted exposure of controls has always taken place but as test articles become more potent the avoidance of contamination becomes increasingly more important.

Scrupulous attention to detail in the analytical laboratory is vital as control contamination may also take place in that environment, but this poster focuses on the strategies that may be employed in the vivarium to avoid the exposure of control animals to test article.

Described here are a number of measures that may be taken, including variations in housing and husbandry techniques as well as procedural rigour. It is important to ensure that these strategies are put in place prospectively to avoid cross contamination, rather than as a remedy after detection. In addition to the evident risks to study integrity, worker safety must always be considered and many of the techniques described also serve to protect the vivarium staff.

2301 AMBULATORY CONTINUOUS INTRAVENOUS INFUSION MODEL FOR PREGNANT DUTCH BELTED RABBITS.

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Continuous intravenous infusion (CI) is a common route of clinical administration. An ambulatory CI model for pregnant Dutch Belted rabbits [(DB) SPF] was developed as an alternative to the tethered CI system. The ambulatory and tethered systems were evaluated for potential effects on pregnancy, including viability, growth and development of the embryo and fetus. Rabbits (25/group) were surgically prepared with an indwelling catheter implanted in the femoral vein. A control group of 10 rabbits was not surgically manipulated or treated. All rabbits were inseminated with semen from untreated males. Infusions were delivered using the AVA Biomedical Cath-in-Cath™ Infusion System. Catheterized rabbits were fitted with Lomir™ infusion jackets. PCA50 infusion pumps were used for the ambulatory group, and sterile plastic syringes and calibrated Razel™ syringe pumps were used for the tethered group. Rabbits received 0.9% NaCl for injection at a target rate of 0.4 mL/kg/hr continuously from GD 7 to 20; actual mean delivery rate was 0.39 mL/kg/hr for both infusion groups. There were no significant effects on maternal body weight or food consumption during the infusion period. Pregnancy rates in the control, ambulatory and tethered groups were 70%, 96% and 88%, respectively, compared to 90±5% in WIL's historical control database for naturally mated DB rabbits. Mean numbers of viable fetuses per litter were 6.0, 5.3 and 5.0 in each respective group, with mean postimplantation losses of 10.7%, 19.0% and 26.3%. Numbers of corpora lutea per doe were similar across groups. Fetal weights were also similar across groups. External malformations were present in 0/36, 2/128 and 2/104 fetuses in the control, ambulatory and tethered groups, respectively. The ambulatory CI model was found to be comparable to the tethered CI system, with no significant effects on pregnancy or embryo-fetal development relative to the control group; however, the ambulatory system was less labor-intensive.

2302 OPTIMIZING CELL LINES FOR DETECTION OF DRUG-INDUCED MITOCHONDRIAL IMPAIRMENT.

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Unanticipated xenobiotic-induced organ toxicity continues to undermine the risk-to-benefit ration of many pharmaceuticals. For example, statins are associated with skeletal, but not heart, rhabdomyolysis, thiazolidinediones with hepatotoxicity, and doxorubicin with cardiac, but not skeletal muscle toxicity. In all three instances, mitochondrial dysfunction has been implicated. The inability of many pre-clinical screens to predict such toxicity prompted us to test the notion that organ-specific cells lines could be optimized to reveal drug-induced toxicity. Here we studied a heterogeneous collection of 73 drugs and compounds associated with hepatotoxicity, rhabdomyolysis and cardiotoxicity. Effects of these drugs were examined in three cell lines: HepG2 (human hepatoma), C2C12 (mouse skeletal myoblast) and H9C2 (rat heart embryonic myoblast). Because many of the selected drugs are known to undermine mitochondrial function, all three cell lines were also cultured with media where glucose is replaced by galactose. Galactose yields no net ATP from glycolysis, thereby forcing the cells to rely on mitochondrial respiration for ATP production. Drugs with toxicity mediated primarily via mitochondrial effects are therefore more toxic to cells grown in galactose. In HepG2 cells, O2 consumption doubles within 1 hr of transfer to galactose and remains at this level indefinitely, while extracellular acidification rate (ECAR; a reflection of lactate efflux) is acutely reduced three-fold, and after several months in galactose culture is further repressed to 20% initial value. In contrast, C2C12 cells accelerate respiration by only 60% both acutely and long term, and respiration in H9C2 is not altered after substrate change. ECAR is halved in C2C12 cells and reduced 3-fold in H9C2 cells. Respiration by the latter two cell lines never exceeded that shown by HepG2 cells grown in glucose. It remains to be seen whether the higher aerobic poise in HepG2 cells will facilitate detection of drug-induced mitochondrial impairment.

2303 A NOVEL IMMUNOHISTOENZYMOGRAPHY APPROACH FOR THE *IN SITU* ASSESSMENT OF DRUG-TARGET INTERACTIONS.

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A major challenge in mechanistic toxicology is to assess the functional relationships between drug-target interactions and target organ toxicity. Here we describe a novel quantitative functional approach for simultaneous in-situ assessment of pharmacological target activity and localization in tissues obtained from preclinical animal models. Using specific inhibitors of the prolyl dipeptidyl peptidase (DPP) enzyme family as a model, we have combined an enzyme activity assay (histoenzymography) with immunohistochemical localization of DPP proteins on the same tissue section. DPP enzymes catalyze the release of fluorescent AFC from the synthetic substrate Ala-Pro-AFC. When performed on histological slides, the resultant signal of this reaction can be localized and quantified using confocal microscopy. Concomitant immunohistochemical staining allows for the unequivocal assignment of enzyme activity to specific DPP proteins. DPP inhibitors with varying se-

lectivity profiles can be used to discriminate between DPP4 and DPP8/9 enzyme activity. Rat kidney tissue sections were incubated with the Ala-Pro-AFC, fixed and immunostained for DPP4 and DPP9 prior to image acquisition. Our results show that rat kidney contains high levels of DPP activity that co-localizes with DPP4 protein in the proximal tubules and could be inhibited by pre-incubation with vildagliptin. A distinct pattern of localization was seen for DPP9. This immunohistoenzymography method can be applied to both frozen and formalin-fixed tissue samples, the latter providing superior spatial resolution. The ex vivo application of this approach to tissue samples from preclinical toxicity studies should provide novel insights into the mechanisms of action of DPP inhibitors in specific sub-cellular domains. Moreover, it can be applied to additional classes of enzymes for which suitable fluorescent synthetic substrates are available.

2304 BEAT-TO-BEAT ELECTRICAL ALTERNANS IN GUINEA PIG LANGENDORFF HEARTS AS A PREDICTOR OF CARDIOTOXICANTS.

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Beat-to-beat alternans of cardiac electrical activity have been proposed as an immediate precursor of ventricular arrhythmias and also used as a proarrhythmic marker in both clinical and experimental settings. In this study, the electrical alternans of electrocardiogram (ECG) and monophasic action potential (MAP) in isolated guinea pig Langendorff hearts were evaluated utilizing six reference compounds known to be either torsadogenic or devoid of arrhythmogenesis in the clinic. Ascending concentrations at therapeutic exposure levels and above were tested for each compound (n=4). Electrical alternans were elicited by pacing the heart at 3-5 fold threshold voltage via the left ventricle epicardium with a programmable stimulator. The stimulus protocol consisted of two short trains that repeated seven times: a preconditioning train of 30 pulses at a fixed basic cycle length (BCL) of 250 ms followed immediately by a testing train of 30 pulses at a variable BCL beginning at 180 ms through 120 ms in 10 ms decrements. The results revealed the following: 1) When electrical alternans occurred, the beat-to-beat changes in T-wave amplitude were much more prominent than those seen in MAP duration. Additionally, T-wave alternans were often observed while MAP alternans were absent; 2) Compounds that possess arrhythmogenic potential in the clinic, such as terfenadine, bepridil and doxorubicin, significantly induced electrical alternans at therapeutically relevant concentrations; 3) Although the electrical alternans often occurred concurrently with prolonged QT/MAP duration, the electrical alternans could present without any changes in QT/MAP duration; and 4) Torsade de Pointes was reproducibly induced in hearts perfused with doxorubicin, a widely-used anticancer agent with well-established cardiotoxicity. These findings were consistent with those obtained from Roche development compounds, suggesting that the beat-to-beat electrical alternans may be a sensitive and robust biomarker in predicting arrhythmic cardiac events potentially superior to the currently ubiquitous models of hERG/QT liability.

2305 THIRTEEN-WEEK VEHICLE FEASIBILITY STUDY BY CONTINUOUS INTRAVENOUS INFUSION IN THE MONKEY.

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Successful conduct of regulatory toxicity studies by continuous intravenous infusion in monkeys is dependent on management of potential catheter tract infections and choice of a suitable vehicle. The study objective was to perform 13 weeks of intravenous infusion of saline or 50 % PEG in monkeys using technical procedures to manage infection associated with the presence of an indwelling catheter. The incidence of inflammatory reactions was compared between the two regimes. Twelve purpose-bred cynomolgus monkeys were divided into two groups of three males and three females. The animals were anaesthetised and surgically implanted with a catheter into the posterior vena cava via the femoral vein. The catheter was attached to a delivery system via a tether and swivel joint. One group of animals received physiological saline and the other PEG 400 at a flow rate of 24 ml/kg/day for up to 12 weeks. Standard toxicology endpoints were measured. A dedicated team of technicians and scientists was used to provide comprehensive antibiotic/analgesic protocols pre/post surgery and meticulous cleanliness in all technical procedures. Three saline infused animals completed 13 weeks of treatment. The remaining animals were terminated in week 12 for technical/animal health reasons. One animal treated with PEG completed the 13 weeks of treatment; the remaining animals were removed by week 5 for technical/animal health reasons. Blood analysis revealed low red cell and high white cell counts and high globulin and C-reactive protein concentrations in animals treated with PEG. Microscopically, PEG infusion was associated with fibrinous thrombi, vein wall necrosis and/or perivascular chronic inflammation. These lesions were not present in saline infused animals. In conclusion, intravenous infusion of saline in the monkey is possible for up to 12/13 weeks with stringent attention to aseptic technique and well structured antibiotic therapy/analgesia protocols. The use of non-aqueous vehicles such as PEG renders long term infusion impractical.

2306 A CARDIOVASCULAR SAFETY ASSESSMENT PARADIGM FOR THE EARLY IDENTIFICATION OF PROARRHYTHMIC AND TORSADAGENIC COMPOUNDS.

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The early identification of potential cardiovascular effects, as well as detection of drug-induced arrhythmias including torsades de pointes is critical to the non-clinical safety evaluation of potential new pharmaceuticals. However, there is currently no one single in vitro or in vivo assay that can determine if a compound that blocks the hERG channel will induce arrhythmias. Here we describe the identification of a torsadagenic compound during the initial preclinical safety testing and the approach now being used for the early identification of proarrhythmic effects. A pharmaceutical lead development candidate with a hERG IC50 of ~12 μM was found to cause: prolonged QTc, increased QRS, tachycardia, and myocardial degeneration at plasma exposure levels greater than 100X the estimated efficacious Cmax. During a subsequent repeat dose cardiovascular investigation the compound was observed to induce multiple instances of arrhythmias, including torsades de pointes with subsequent ventricular fibrillation in some instances. A histopathological evaluation did not find any evidence of microscopic effects. Additional testing for mitochondrial and direct cardiovascular toxicity revealed no evidence of effects, and it showed moderate potency against the sodium channel when tested at a physiological frequency. The compound also caused instability, QT/MAPD prolongation, triangulation, increased QRS duration/AV block, and ventricular ectopic contractions in the isolated guinea pig heart (Langendorff model). It was concluded that the effects on cardiovascular conduction preceded and likely induced the myocardial degeneration. In order to identify the effects at an earlier stage in the development process we test compounds from this class against IKr and sodium channels followed by the isolated heart, and then finally in a dedicated repeat dose in vivo cardiovascular study prior to conducting repeat dose toxicology studies.

2307 EFFECTS OF INSULIN ON THE QT INTERVAL IN ANESTHETIZED DOGS.

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The prolongation of QT interval was reported in the healthy subjects using insulin clamp technique. However, the close relationship between the insulin action and the QT interval has not been clarified. Therefore, the effects of hypoglycemia induced by intravenous injection of insulin on the electrocardiogram were investigated in time-course manner using anesthetized dogs. Four beagle dogs were kept under prolonged anesthesia with inhalation of halothane, and were intravenously injected with rh-insulin (Japanese Pharmacopoeia) solution at a dose of 0.1 mg/kg (2.6 IU/kg). Measurement of electrocardiogram (ECG) parameters was performed on all animals just before dose (pre), and from 5 minutes post-dose until 4 hours. Along with the ECG measurements, plasma samples were obtained form the cephalic vein to determine glucose and electrolytes. The QT interval and the QTc (calculated by van de Water formula) were significantly prolonged from 5 minutes post-dose (prolonged approximately 26% and 21% from the respective pre-values), and remained prolonged for 4 hours post-dose. In addition, two animals showed a high frequency of ventricular premature beats between 1 and 1.5 hours post-dose. The plasma glucose level was significantly decreased from 15 minutes post-dose, and it became approximately 20% of the pre-value at 1.5 hours post-dose, and the minimum value was observed at 2.5 hours post-dose (approximately 16% of the pre-value). Plasma potassium level showed a similar change to that of the glucose; significant decrease from 15 minutes post-dose and the minimum value at 2 hours post-dose (approximately 57% of the pre-value). These results suggested that the excess dosing of insulin caused prolongation of QT interval and QTc. In addition, the high frequency of ventricular premature beats might be related to the prolongation of QT interval.

2308 NILOTINIB PRODUCES NO DETECTABLE CARDIOTOXICITY AFTER 39 WEEKS OF ORAL (GAVAGE) ADMINISTRATION TO CYNOMOLGUS MONKEYS.

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Nilotinib (Tasigna®, AMN107) is a highly-potent, selective tyrosine kinase inhibitor specifically designed to overcome many Bcr-Abl mutations known to cause imatinib resistance. Questions of cardiac toxicity have been raised recently with

other tyrosine kinase inhibitors including one structurally related to nilotinib. Young adult Cynomolgus monkeys were administered 0, 30, 200, or 600 mg nilotinib in 0.5% hydroxypropylmethylcellulose once daily by oral gavage for 39 weeks with 4-week recovery. In-life observations included periodic eye exams, body weight and food consumption monitoring, and clinical pathology analyses. Electrocardiography was performed under ketamine sedation during pre-test acclimation and at least 2 hours post-dose in study weeks 13, 26 and 39. Serum concentrations of AMN107 were determined on days 1/2 and during weeks 4, 13, 26, and 39 of dosing. Animals were euthanized for necropsy and histopathology at the end of dosing or after the recovery period. Formalin-fixed heart samples were post-fixed for ultrastructural analysis from 4 controls and 8 monkeys that received 600 mg/day for 39 weeks. No in-life signs or clinical pathology parameters were indicative of cardiotoxicity, no arrhythmias or electrocardiographic abnormalities were seen, and no heart weight, microscopic or ultrastructural changes were seen in the heart of monkeys with mean AMN107 exposures ($AUC_{0\text{-}24hr}$ ± SD)of 26200 ± 6420 (males) or 24400 ± 10300 ng-h/mL (females) after 39 weeks of dosing. These results indicate that nilotinib produced no detectable cardiotoxicity after 39 weeks of oral administration to Cynomolgus monkeys.

2309 TOXICOLOGICAL EVALUATION OF BMS-747158, A PET MYOCARDIAL PERFUSION IMAGING AGENT.

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BMS-747158-02 is an [18F]-labeled pyridazinone that binds potently to mitochondrial complex I and is being developed as a myocardial perfusion imaging agent. During radiolabeling, a hydroxylated analog (BMS-747159) is formed as well as the fluorinated product. Toxicology studies were conducted with both [19F]BMS-747158 and BMS-747159 to support the clinical use of BMS-747158 as a PET agent. Intravenous administration of either BMS-747158 alone as a single dose (≤ 100 <μ>g/kg) in rats or as a 1:10 (w/w) mixture with BMS-747159 (≤ 5/50 <μ>g/kg) in rats and dogs produced no clinical signs and no gross or histopathological changes. Acute transient clinical signs (rapid and labored breathing, decreased activity, hunched posture, prostration including salivation, defecation, emesis vocalization and urination in dogs) were observed at high dose levels of BMS-747158 alone (≥ 200 <μ>g/kg) and the mixture with BMS-747159 (≥ 5/50 <μ>g/kg). At very high dose levels of BMS-747158 alone (≥ 200 <μ>g/kg) or in combination with BMS-747159 (≥ 20/200 <μ>g/kg) caused lethality in some instances in rats. Repeat dose (28 day) toxicity studies with the BMS-747158/BMS-747159 mixture caused no effects (mortality, clinical signs, ophthalmoscopic, electrocardiogram [dogs], clinical and gross pathology or histopathology) at (≤ 15/150 <μ>g/kg) in the rat and at (≤ 5/50 <μ>g/kg) in the dog. Based on a clinical dose of under 0.07 <μ>g/kg of BMS-747158, there would be a large margin of safety. Measurement of plasma concentrations of BMS-747158 and BMS-747159 in the rat and dog indicated rapid blood clearance from circulation, short half-lives, a linear increase in maximum concentration (Cmax), and area under the curve (AUC). A large volume of distribution was evident and no gender differences in exposure or systemic accumulation for both species with respect to dose. Overall, acute and repeat dose toxicity studies with BMS-747158 demonstrate transient and dose-related increase in clinical signs in rats and dogs.

2310 TOXICITY OF NORWALK VLP VACCINE FOLLOWING REPEATED INTRANASAL ADMINISTRATION TO NEW ZEALAND WHITE RABBITS.

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Norovirus infection is the most common cause of acute gastroenteritis that can result in severe clinical outcomes in the elderly, young, and hospitalized. The Norovirus vaccine is a dry powder formulation based upon virus like particles (VLPs) that mimic the virus but are not capable of causing illness. The purpose of the present study was to evaluate the potential toxicity of the vaccine following three intranasal doses to rabbits. Rabbits were randomly assigned to three groups (10 rabbits/sex/group). Animals in Group 1 were not dosed. Animals assigned to Groups 2 and 3 were administered the placebo and vaccine, respectively on Days 1, 22, and 43. Mortality, clinical and cageside observations, body weights, body weight changes, food consumption, body temperature, ophthalmology examinations, clinical pathology, gross pathology, organ weights, and histopathology were evaluated. There were no adverse effects on mortality, clinical or cageside observations, body weights, body weight changes, food consumption, body temperature, ocular examinations, gross pathology, absolute or relative organ weights, and urinalysis parameters. Higher mean and individual total protein and globulin values and lower mean and individual animal albumin/globulin ratio values were noted in placebo-treated animals throughout the study, and were considered to represent a polyclonal activation of B lymphocyte populations. Elevated absolute monocyte values for all animals receiving placebo and vaccine was also observed throughout the study. Microscopic findings consisted of varying degrees of inflammatory infiltrates, either within the lamina propria of nasal turbinates or free within the nasal passages, and/or hemorrhage in the nasal passages of rabbits in the placebo- and vaccine-treated groups. Female rabbits receiving placebo also had some evidence of lymphoid hyperplasia. These lesions are consistent with an immunologic reaction, were limited in nature, and completely resolved following the recovery phase.

2311 DRUG-INDUCED PHOSPHOLIPIDOSIS: RELATIONSHIPS BETWEEN HISTOLOGICAL CHANGE, TISSUE INJURY AND DRUG ACCUMULATION.

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Drug-induced phospholipidosis (PLD) is characterized by intracellular accumulation of phospholipids and ultrastructural membranous lamellar inclusions in lysosomes. In vivo administration of cationic amphiphilic drugs to animals can cause PLD. The general consensus is that PLD is an adaptive response rather than a toxicological manifestation; however, additional studies are required for a better understanding of the functional consequences of PLD (Reasor et al., Expert Opin. Drug Saf. 5, 567, 2006). Our study was conducted to investigate the toxicological significance of drug-induced PLD. Rats were treated with cationic amphiphilic drugs by gavage for four days. Drugs tested were ketotifen, promazine, homochlorcyclizine, diltiazem, labetalol, chlorpromazine, maprotiline, telithromycin and amiodarone. Grading of PLD (the extent of increase in membranous lamellar inclusions by transmission electron microscopy) in lung and liver, blood biochemistry, and measurement of blood and tissue drug levels were performed. All nine drugs caused PLD. Slight to mild PLD was observed mainly at doses from 30 to 100 mg/kg; moderate to severe PLD at 500 mg/kg. The plasma AUC levels of drugs causing slight PLD in rats were comparable to those in humans at therapeutic dose levels. No significant elevations of serum ALT levels (>3-fold increase vs. control) were observed even in cases of severe liver PLD. In slight to mild PLD, drugs did not accumulate in the liver or lung. However, in moderate to severe PLD extensive drug accumulation (100-800 μg/g) was observed in these tissues. Present results suggest that mild PLD graded by transmission electron microscopy does not indicate a toxicological manifestation, while moderate to severe PLD accompanied with extensive drug accumulation in tissues may be concern of toxicological significance for a drug's long term safety.

2312 INCREASED EFFICIENCY OF PROCONVULSANT SCREENING THROUGH STRATEGIC USE OF ELECTROCONVULSANT MODELS.

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Proconvulsant liability can be detected by a number of methods, including observation of motor behaviors and sensitivity to standardized seizure precipitants. A previously described behavioral observation model detected convulsant activity in compounds from two projects: 5HT receptor antagonist with chemistry-related toxicity, and an opioid receptor agonist with target-related toxicity. Two electroshock (ES) models, increasing current electroshock (ICES) and 6Hz minimal electroshock (6Hz minES) were evaluated as proconvulsant screens. Convulsant and nonconvulsant proprietary compounds were assessed for each project. ICES reliably detected lowered seizure threshold in mice treated with proprietary 5HT antagonist compounds at doses that evoked motor signs of seizure, but did not detect a decrease in seizure threshold in convulsant opioid agonist-treated mice. Conversely, 6Hz minES reliably detected proconvulsant liability in mice treated with convulsant proprietary opioid agonists (~7X sensitivity over the original model), but not in those treated with convulsant 5HT antagonists. Both ES models increased time efficiency ~10X, and post-precipitant duration per subject was decreased 5- or 60-fold compared to the behavioral observation model. Both were predictive of compound convulsant liability in other species. We conclude that neither ES model can be applied uniformly across all projects, but either ES model may serve as sensitive, efficient screen against proconvulsant liability when validated for specific target- or chemistry-related toxicity. This research was funded by AstraZeneca Pharmaceuticals.

2313 TEMPORAL RELATIONSHIP OF PHOSPHOLIPIDOSIS AND MITOCHONDRIA TOXICITY IN PRIMARY RAT CEREBELLAR CULTURES.

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Cationic, amphiphilic drugs (CADs) are reported to cause local or systemic phospholipidosis (PLD) after chronic exposure in preclinical studies. CNS and systemic PLD is a liability for psychiatry projects as neurodegeneration is hypothesized to

occur secondarily to PLD with a small number of phospholipogenic drugs, including perhexiline and chloroquine. Many neurodegenerative compounds are also mitochondrial toxicants and some lipid storage disorders are associated with neurodegeneration. In view of this, the temporal occurrence of phospholipid accumulation and mitochondrial effects was investigated. Primary cultures from neonatal (P2-P5) rat cerebella were established and used for in vitro PLD and mitochondrial toxicity analyses. A previously established in vitro PLD assay using fluorescent-tagged phosphatidylethanolamine (NBD-PE) in mouse splenocytes was adapted to the primary cerebellar cultures. Similar rank order phospholipogenic potency responses to CADs were observed between these two cell contexts. Phospholipogenic responses and effects on mitochondrial polarization were then determined for classic CADs in cerebellar cultures over a 24 hour time period. JC-1 was used as a mitochondrial polarity indicator. Comparison of NBD-PE and JC-1 curves indicated that the time frame of both PLD and mitochondrial effects overlapped, indicating mitochondrial toxicity was not secondary to phospholipidosis in this model and that direct mitochondrial toxicity may be invoked as a mechanism of neurodegeneration.

2314 14-DAY SODIUM 2, 2-DIMETHYLBUTYRATE (ST-20) NEURO/GENOTOXICITY STUDY IN RATS.

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ST-20 (NSC-D741804) is a potential therapeutic agent for β-thalassemia at estimated human doses <14 mg/kg. Neuro/genotoxicity were studied in SD rats (five/sex/dose) at gavage dosages of 200, 600, or 1000 mg/kg (5 mL/kg), once daily, for 14/15 days with a recovery period (Day 29). Clinical findings included ataxia (F), rough coat/thin appearance (M), and decreased BW (M) at 1000 mg/kg. FOB deficits (F > M) included decreased body tone (M/F, 600 and 1000 mg/kg), rectal temperature (F, 600 mg/kg; M/F, 1000 mg/kg), emotional reactivity (F, 600 and 1000 mg/kg), neuromotor-neuromuscular activity (F, 600 mg/kg; M/F, 1000 mg/kg, as exhibited by a deficit in visual/tactile placing accuracy, ataxia, hindlimb dragging, and decreased grip strength), and rearing (F, 1000 mg/kg). ST-20 (Day 15) decreased RBC, Hgb, Hct, MCV, and MCHC, increased reticulocytes and erythrocytic Pappenheimer Bodies (M/F, 600 and 1000 mg/kg), decreased TP/globulin, increased AG ratio (M/F, 600 and 1000 mg/kg), and decreased glucose (M/F, all groups). On Day 29, clinical signs, hematology and serum chemistry showed a reversible trend or complete recovery. Micronucleated PCEs increased slightly (M, 1000 mg/kg) but its biological relevance is questionable. Group mean liver-to-body weight values (percent of control) increased (M/F, 600 and 1000 mg/kg). Centrilobular hepatocellular hypertrophy (Day 15 but not Day 29) was observed (M, 1000 mg/kg), which was interpreted to be a compensatory response in the absence of increased serum ALT, AST, ALP, or GGT. The NOAEL is 200 mg/kg in male and female SD rats following gavage administration of ST-20 for 14 consecutive days. The presence of premonitory signs, reversibility, and a reasonable safety margin indicate ST-20 is a promising candidate for clinical evaluation in this serious disorder. (Supported by NCI contract N01-CM-42200, NIH grants R01 DK-52962, R42 HL-62715 and NHLBI and NIDDK under NIH-RAID Pilot Program).

2315 PEDIATRIC NON CLINICAL SAFETY OF LEVETIRACETAM IN COMPARISON TO OTHER AEDS.

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Epilepsy is a serious, commonly occuring disease, affecting ~2% of the general population which requires a chronic treatment with antiepileptic drugs (AEDs), often in combination. Teratogenicity of some of the 'older' AEDs (valproic acid, VPA; phenytoin, PHT; carbamazepine, CBZ; diphenylhydantoin, DPHT) has been recognized and adverse effects on cognitive development are being suggested. In view of this latter issue, safety of these agents is of particular concern in the case of children whether they have been treated in utero or per se. To minimize these risks, information on the safety profile of AEDs for pediatric use is essential to properly balance the need for efficacy to patient and the potential for long term side effects. This poster will focus on the animal and human data currently available regarding the potential to induce behavioral defects, comparing a 'new' AED, Levetiracetam (LEV) to other AEDs. In the regulatory package of reproductive studies performed to market LEV, two types of studies assess the potential behavioral effects of LEV on the development of rat pups: the peri and post natal development study where fetuses/pups were exposed in utero and/or through the mother's milk, and juvenile studies where pups/young adults were directly treated. In those studies no behavioral effects (rotarod, open field, passive avoidance, water maze and auditory startle habituation) were observed in the pups up to 1800 mg/kg/day. This dose represents for the mother, on a body weight basis, more than 30 times the maximum recommended oral dose in humans (3000 mg/day). In contrast, VPA, PHT, CBZ or DPHT induced long term behavioral toxicity in rats or mice assessed through circling test, rotarod, open field, maze tests or auditory startle test. This is consistent with the increasing literature reporting adverse effects on behavioral development of children with these same 'old' AEDs. The favorable profile of LEV compared to other AEDs in rat non-clinical studies assessing the potential for long term side effects may be one of many key factors to consider when choosing a treatment in pediatrics.

2316 COLLECTION OF CEREBROSPINAL FLUID BY DIRECT PUNCTURE OR BY SURGICALLY IMPLANTED PORTS IN MONKEYS.

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Collection of cerebrospinal fluid (CSF) is often performed in preclinical studies using the intrathecal, epidural or intracerebroventricular routes of administration, to assess CSF drug levels and kinetics. Collection of CSF (non-terminal) is a challenging technical procedure and in monkeys can be done by direct puncture or via a surgically implanted catheter. The success of both techniques and selection of the preferred approach will depend on the number of collection points over a given period and the volume of collection. Consideration for the inclusion of additional animals may be required to obtain a minimum number of samples.

In our laboratories, samples were collected from the cisterna magna by direct puncture under anesthesia or via an implanted catheter in the cisterna magna connected to a subcutaneous access port in conscious monkeys. CSF samples were obtained over up to a 29 day period. Rate of success was variable with both techniques (approximately 70% for direct puncture; 75 to 100% with access ports). Several samples were analyzed for bacteriology, red blood cell and white blood cell counts. Neurological evaluations were conducted on all animals to evaluate the impact of CSF collection. Presence of red or white blood cells was detected in some samples.

For limited sampling, collection by direct puncture into the cisterna magna can be successfully used, however anesthesia is required and therefore can be limiting. The use of implanted catheters with subcutaneous access ports provide a viable alternative for collection over multiple time points, however, obstruction/blockage of the catheter may occur. Procedures to re-establish catheter integrity have been achieved however with variable success.

It can be concluded that collection of cerebrospinal fluid by direct puncture or by surgically implanted ports is feasible for at least 29 days with a success rate ranging from 70 to 100% for either method. The choice of the method of collection should be defined by the frequency of the sampling.

2317 A PERFORMANCE STANDARD FOR DEVELOPMENT OF THE MODIFIED IRWIN TEST AS PART OF A NEUROLOGICAL ASSESSMENT AND A FUNCTIONAL OBSERVATIONAL BATTERY (FOB) IN CD-1 MICE.

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As part of the safety pharmacology core battery, as stated by the US FDA ICH guidelines, a compound's potential for neurotoxicity must be assessed. This can be achieved by evaluating peripheral and central nervous system integrity via a Functional Observational Battery (FOB), or a Modified Irwin's Test. The purpose of this work was to validate the procedures surrounding the conduct of the FOB and Modified Irwin's Test in mice in our laboratories. Positive control drugs (Diazepam, Amphetamine and Physostigmine & Atropine) were selected to produce neuropharmacological effects which increase or decrease scoring during the Modified Irwin's Test and/or FOB. As a first step, 6 technicians, blinded to animal identification, were required to evaluate the qualitative parameters of the FOB (Modified Irwin's Test) which included Open-Field, Manipulative and Physiological Evaluations. Technicians were required to each assess, in random order, 4 CD-1 mice (2/gender) from each group. Scoring was evaluated against the internal performance standard and served to assess the inter-analyst variability. As a second step, the quantitative elements of the FOB were evaluated, including grip strength, landing footsplay, rotarod and figure-8 maze. An additional group (Buprenorphine) was added to allow validation of the identification of analgesic effects. The quantitative elements of the FOB were not included in the assessment of inter-analyst variability as the data were not subjective. Results obtained during the blinded evaluation of qualititative parameters demonstrated that scoring of these parameters was consistent and comparable between individual technicians. In addition, the data demonstrated that the drugs and dose levels chosen produced the expected pharmacological effects, and were appropriate to validate the qualitative and quantitative parameters of the FOB, without any gender differences. The validation was therefore considered to be successful.

2318 NEUROBEHAVIORAL EFFECTS OF D-AMPHETAMINE AND CARBARYL IN THE VALIDATION OF SAFETY PHARMACOLOGY CENTRAL NERVOUS SYSTEM TESTING METHODS IN SPRAGUE DAWLEY RATS.

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A regulatory-compliant method was developed to assess the neurological safety of novel therapeutic agents under the ICH S7A guideline and regulations for safety pharmacology. A Functional Observational Battery (FOB) was designed to examine 35 neurobehavioral endpoints of the Sprague-Dawley rat after administration of CNS altering substances (d-amphetamine by intraperitoneal injection, or carbaryl by oral gavage). The FOB observations were classified into functional domains such that activity and excitability, neuromuscular integrity, response to sensory stimuli, autonomic nervous system activity and physiological endpoints were evaluated. The study was comprised of eight groups of 5 male and 5 female rats that received either a vehicle control (0.9% saline or corn oil) or d-amphetamine at doses of 1, 4, or 8 mg/kg or carbaryl at doses of 10, 30 or 100 mg/kg. Increases in activity, arousal, stereotypic behavior, difficulty in handling, impaired gait, increases in rearing, and manipulative tests were observed with d-amphetamine administration up to 8 mg/kg. Carbaryl administration up to 100 mg/kg showed decreases in body temperature, activity and arousal, along with observations of tremors, muscle fasciculation, constricted pupils and impairments in manipulative observations. The results were consistent with expected pharmacological actions of d-amphetamine or carbaryl exposure with effects observed across the functional domains. The statistically significant changes along with observable dose-related trends provides sufficient evidence to support that this neurobehavioral assessment produces reliable data and is effective in detecting changes to neurologic function in the rat model.

2319 MOTOR ACTIVITY AND FUNCTIONAL OBSERVATIONAL BATTERY IN RATS TREATED WITH EMD 509847 AND CNS REFERENCES.

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The functional observational battery (FOB) is a systemic evaluation of nervous system function in the rat, including more than 30 parameters across the autonomic, neuromuscular, sensomotoric and central nervous domains. Motor activity measurement followed by the FOB were evaluated in a pharmaceutical development compound against six different known CNS active references as part of the safety pharmacology "core battery".
EMD 509847, a Factor Xa inhibitor, was administered orally to 5 Wistar rats/sex/group as a single dose of 150 mg/kg. A control group received the vehicle, 0.25% methocel. Six reference groups were dosed with 20 mg/kg (p.o.) caffeine, 3 mg/kg (i.p.) haloperidol, 1 mg/kg oxotremorine (i.p.), 2 mg/kg amphetamine (i.p.), 90 mg/kg pentetrazole (s.c.), and 2 mg/kg 8OH-DPAT (s.c.). Motor activity was measured for 1 hour, the rats' behaviour was tested in a FOB thereafter.
Motor activity: No differences between EMD 509847 treated animals and vehicle controls were observed. Caffeine, amphetamine and 8OH-DPAT increased the motor activity, whereas haloperidol and oxotremorine decreased it. No significant differences were seen with pentetrazole.
FOB:
Effects on autonomous domain parameters were seen with EMD 509847, haloperidol, pentetrazole or 8OH-DPAT. Changes in the neuromuscular domain were observed after caffeine, haloperidol, amphetamine, or pentetrazole treatment. Haloperidol, pentetrazole and 8OH-DPAT affected the sensomotoric domain. Caffeine, haloperidol, amphetamine, oxotremorine, pentetrazole and 8OH-DPAT changed central nervous domain parameters.
Conclusion:
EMD 509847 did not induce any substantial changes of motor activity or behaviour. The CNS references caffeine, haloperidol, oxotremorine, amphetamine, pentetrazole, and 8OH-DPAT caused a number of changes in various domains of the central nervous system. Therefore, motor activity measurement combined with a FOB is a useful tool to investigate effects on the central nervous system of new pharmaceutical compounds.

2320 THE SIGNIFICANCE OF SKIN DISTRIBUTION OF DRUGS FOR THE ELICITATION OF PHOTOTOXIC REACTION.

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Phototoxicity is defined as a toxic response that is induced by drug administration and subsequent exposure to light. It is well known that many approved drugs including quinolone antimicrobials can induce photoirritation in humans following oral administration. In the present study, the in vitro 3T3 NRU phototoxicity test and an in vivo mouse test were performed using known phototoxic and non-phototoxic drugs to assess the predictability of these test methods for clinical effects. The in vitro phototoxicity test was performed according to OECD guideline No. 432. For the in vivo test, female BALB/c mice were treated with each drug by gavage. Commencing 30 min after dosing, the mice were exposed to simulated sun light (SOL500, Honle UV Technology) at an intensity of 20 J/cm2 for 120 min. The ears of mice were evaluated for erythema and auricular thickness 1, 24, 48, 72 and 168 h after the completion of irradiation. Plasma and auricular tissue concentrations of the drug were measured 1, 2, 4 and 24 h after dosing. The phototoxic drugs, sparfloxacin and lomefloxacin, exhibited phototoxicity in both in vitro and in vivo tests. The plasma AUC levels of sparfloxacin and lomefloxacin at doses that caused photoirritation in mice were comparable with those at therapeutic doses in humans. Auricular tissue concentrations of these drugs were higher than plasma concentrations (auricular tissue/plasma ratio: 6-9). In contrast, one drug that is not known to be phototoxic in humans showed a positive reaction in the in vitro test but did not exhibit in vivo photoirritation in the mouse test. Auricular tissue concentrations of this drug were low compared with plasma concentrations. In conclusion, for phototoxicity evaluation, an in vitro phototoxicity test is useful for hazard identification of drugs; however, to predict clinical phototoxic effects the in vivo test system in which skin distribution of drugs affects the outcome is considered to provide more reliable assessment.

2321 *IN VITRO* ASSAYS FOR EVALUATING THE ULTRAVIOLET A (UV-A) INDUCED DAMAGE IN CULTURED HUMAN RETINAL PIGMENT EPITHELIAL CELLS.

L. Lee. *Pfizer Global R&D, San Diego, CA.* Sponsor: W. Yvonne.

Retinal pigment epithelial cell exposure to solar and occupational ultraviolet (UV) radiation induces cell death, leading to photoreceptor dysfunction and ultimately visual impairment. We established an in vitro model to evaluate UV-A-induced damage of cultured human retinal pigment epithelial cells. Cultured human retinal pigment epithelial (ARPE-19) cells were exposed to UV-A (wave length = 365nm) radiation at 5 J/cm2 for 1 hour then allowed to incubate for 24h. Cytotoxicity was measured as a reduction of cellular ATP, using a luciferase luminescence assay. Additional known cytotoxic compounds were also tested similarly. The major advantages of this system are high sensitivity, simplicity, and fast results without the harvesting or separation steps. The results suggested that UVA radiation-induced cell damage can be evaluated by cellular viability. The assay provides a valuable in vitro model for ultraviolet radiation-induced retinal toxicology research.

2322 TOXICOLOGY STUDIES TO SUPPORT CLINICAL DEVELOPMENT OF TREMELIMUMAB, A CYTOTOXIC T LYMPHOCYTE-ASSOCIATED ANTIGEN 4 (CTLA4) BLOCKING MONOCLONAL ANTIBODY.

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Tremelimumab is a fully human anti-CTLA4 monoclonal IgG2 antibody in clinical development for patients with advanced cancer. Tremelimumab has a high affinity for human and primate CTLA4 expressed on the surface of activated T cells but does not recognize mouse, rat, hamster, or rabbit CTLA4. Toxicology studies of tremelimumab were conducted in cynomolgus monkeys using single-dose and 1- and 6-month regimens with weekly dosing. A dose response was observed in systemic exposure, and primate anti-human antibodies were detected in some plasma samples. Toxicities observed were considered related to the pharmacology of tremelimumab and sustained lymphocyte activation. These included loose stools and skin rash. Some animals receiving the highest dose in the 6-month study (50 mg/kg) developed persistent loose stools with weight loss and decreased appetite resulted in cessation of dosing and/or euthanasia. Exposure to tremelimumab induced hyperplasia of lymphoid organs and produced a dose-dependent increase in the incidence and severity of mononuclear cell infiltration or inflammation in most tissues having a spontaneous background incidence of mononuclear cell aggregates. Immunohistochemistry revealed a variety of cell surface markers on circulating lymphocytes and tissue mononuclear infiltrates. After the reversibility period, only minimal inflammation of the salivary gland and skin were observed. Target tissues for toxicity were the gastrointestinal tract, the skin, lymphoid and thyroid tissues, and the hematologic system. Tremelimumab did not elicit developmental toxicity or teratogenicity in a monkey embryofetal development study. The findings from the toxicology studies were generally consistent with the pharmacology of tremelimumab. Furthermore, these findings correlated well with both dose-limiting (diarrhea and skin rash) and non-dose-limiting toxicities observed in clinical trials.

2323 CAECINOGENICITY STUDY OF THE NEW PYRAZOLOPYRIMDINONE DERIVATIVE K-1089 FOR ERECTILE DYSFUNCTION IN F344 RATS.

S. Cha, H. Lee, M. Park, E. Jeong, K. Lee and S. Han. *Korea institute of Toxicology, Daejeon, South Korea.* Sponsor: W. Koh.

The present study was conducted to investigate the carcinogenic potential of K-1089, a new pyrazolopyrimdinone Derivative K-1089 for erectile dysfunction in F344 rats. Groups of 60 males and females were administered by gavage for 104 weeks at daily dose levels of 0, 40, 80, and 160 mg/kg/day. Survival of male and female rats given 160 mg/kg/day was less than vehicle control. In males and females, salivation was noted in the 80 and 160 mg/kg /day groups. The food consumption by 80 and 160 mg/kg/day males and females was higher than that by control. Statistically, significant decreases in red blood cells and hemoglobin and increases in MCV were observed at 80 and 160 mg/kg/day in males, while RBC, hemoglobin, and hematocrit values were decreased at 160 mg/kg and MCV and MCH was increased at 80 and 160 mg/kg/day in females. Males at 80 and 160 mg/kg/day showed increases in proerythroblast, and decreases in total myeloid, mature neutrophil and eosinophil. Females revealed increases in total myeloid at 80 and 160 mg/kg/day, promyelocytes, myelocyte at 160 mg/kg/day, and mature neutrophil at 40, 80, and 160 mg/kg/day. Bone marrow revealed dose-related increases in bone marrow hyperplasia, myelostromal proliferation, and myelostromal sclerosis with lipofuscin (pigment)-laden reticulo-endothelial cells were observed in males at 80 and 160 mg/kg/day and females at 160 mg/kg/day. Spleen showed dose-related increase in the extrameduallary hemopoiesis in males at 80 and 160 mg/kg/day and females at 160 mg/kg/day. At 160 mg/kg, males and females exhibited bile duct hyperplasia and cholangiofibrosis that proliferating bile ducts were surrounded by collagenized connective tissue with lymphoid cell infiltration. Parathyroid hyperplasia and/or cytoplasmic vacuolation were observed in males and females at 160 mg/kg. Based on the results, the treatment-related carcinogenic effect was not observed and the no-observed-adverse-effect level (NOAEL) was considered to be 40 mg/kg/day in both sexes.

2324 DOES ENVIRONMENTAL ENRICHMENT AFFECT TUMOUR INCIDENCE IN RATS?

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Environmental enrichment is the provision of stimuli that promote the expression of species-appropriate behavioural and mental activities in an under-stimulating environment. In this laboratory there is frequent review and improvement of the environmental conditions of all species of laboratory animals while ensuring the scientific objectives of studies are met. In the last few years, in addition to wood chip bedding, aspen wood blocks for chewing have been provided for rats in this laboratory.

The incidence of spontaneous tumours in control animals is used to interpret findings in treated animals in carcinogenicity studies. Therefore relevant and well-controlled historical background data are vital to the interpretation of findings.

In order to determine the impact of environmental enrichment items on spontaneous tumour incidence, a preliminary comparison was performed. Common spontaneous tumour incidences (>5%) from multiple housed (single supplier) Han Wistar rat 2-year carcinogenicity studies where environmental enrichment was provided (n = 4) were compared with those without environmental enrichment (n = 7) by Student's t-tests. In studies with environmental enrichment there was no statistical difference in the incidence of tumours in males or females from those without. In this preliminary investigation there was no impact of environmental enrichment on tumour incidence. As more data become available a further investigation will be undertaken to determine the impact of environmental enrichment on rat and mouse tumour incidences.

2325 TUMORS IN RATS ASSOCIATED WITH IMPLANTABLE MICROCHIP IDENTIFICATION DEVICES.

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Tumors associated with implantable microchip devices can develop in long-term rodent studies. Elcock, *et al.* (Exp. Toxic. Pathol. 2001; 52: 483-491) noted a very low incidence (<1%) of tumors surrounding these implantable devices in two separate chronic bioassays with F344 rats (F344/N or F344/SAS; derived from NIH stock). As a follow up to the previous evaluation, three more-recent long-term studies using Wistar rats (Crl:WI[Glx/BRL/Han]IGS BR) were evaluated for the occurrence of tumors associated with such devices. Like the earlier studies, all three were comprised of both a 1- and 2-year component. Similar tumors were noted in rats sacrificed at two years in two of these studies, at incidences of 0.3 and 1.1%, respectively, but not in the third (the most recent) study. Tumors were not observed in 1-year animals for any of these studies. Results of the previously reported studies have been widely cited as evidence to predict a similar tumor occurrence in other species, including potentially humans. However, these data do not support this contention since these studies were not designed to investigate this issue. This paper will review the findings from five long-term rat bioassays, using two different rat strains, discuss the mechanism for tumor development, and assess the risks associated with tumor formation around implantable microchips versus the benefit of utilizing these devices as a means of animal identification.

2326 SPONTANEOUS HIBERNOMAS IN SPRAGUE-DAWLEY RATS.

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Hibernomas are rare neoplasms originating in brown adipose tissue of humans and other animal species including laboratory rodents. Background incidence values for these tumors in rats are generally accepted as being <0.1%. In several recent carcinogenesis bioassays conducted at our laboratory with Sprague-Dawley rats, benign and malignant hibernomas were markedly increased over background values with an overall incidence of approximately 3%. Tumors were randomly distributed among test article-treated and control groups and all were considered to be spontaneous. Most tumors originated in the thoracic cavity where they were described grossly as soft, tan masses with varying external profiles. Immunohistochemical procedures for Uncoupling Protein 1 (UCP1) confirmed brown adipose tissue as the site of origin. The marked increase in hibernomas in our studies strongly suggests that greater numbers of spontaneous hibernomas may be anticipated in future carcinogenesis studies with Sprague-Dawley rats. The increased potential for hibernomas to arise as spontaneous neoplasms has important implications in studies involving peroxisome proliferator-activated receptor drugs, lipophilic environmental chemicals (e.g., polychlorinated biphenyls), and other molecules or physiologic processes (e.g., β-adrenergic stimulation) that may target brown fat adipocytes.

S 2327 CELLULAR REDOX STATUS AND ZINC SINGALING.

Y. Kang. *Medicine, University of Louisville School of Medicine, Louisville, KY.*

Cellular redox status constantly changes under both physiological and stress conditions. The redox change modifies cellular signaling system, leading to functional adaptation to the physiological demand or the stress imposition. Zinc/cysteine coordination in proteins is sensitive to redox changes and is therefore used in redox switches. There are two major classes of redox zinc switches: the first are redox sensors that undergo conformational changes through zinc release to alter their functions, although the released zinc may not have known functions. The second are redox transducers that release zinc for the purpose of changing the function of other proteins through the released zinc. The function of the redox transducers thus is to convert the redox signal to zinc signals. There is virtually no free zinc in the cell under physiological conditions and zinc availability is tightly controlled through uptake, intracellular sequestration, and controlled trafficking. Metallothionein (MT), as a redox transducer, plays a critical role in the redox sensing and zinc signaling. Oxidation of the sulfur ligands in MT mobilizes zinc, while reduction of the oxidized ligands enhances zinc binding. This MT redox cycle provides an excellent control mechanism for the availability of intracellular zinc. Many zinc proteins are activated or inactivated after receiving zinc under oxidative stress conditions. These zinc proteins are the effectors of zinc signaling and involved in signal transduction, energy metabolism, mitochondrial function, and gene expression. This symposium will present discussions on (1) cellular redox responses under physiological and stress conditions; (2) zinc transporters and the regulation of zinc intracellular movement in mammalian systems; (3) zinc/cysteine coordination in proteins as a sensor to redox signaling and the converting of redox signaling to zinc signaling though MT redox cycle; and (4) regulation of gene expression by zinc-finger transcription factor.

S 2328 REDOX REGULATION OF CELL SIGNAL TRANSDUCTION.

D. P. Jones. *Medicine, Emory University, Atlanta, GA.*

Under both physiological and stress conditions, cellular redox status constantly changes. These changes start from oxidation of redox sensitive molecules, which in turn alter the cellular redox equilibrium, and subsequently affect signal transduction resulting in functional adaptation or alteration of effectors. Our research has

used plasma thiol/disulfide redox states as a means to evaluate oxidative stress in human studies. Results show that the GSH/GSSG redox state and cysteine/cystine (Cys/CySS) redox states are not equilibrated and correlate with different disease risk parameters. In an ancillary study to the NEI-supported Age-Related Eye Disease Study (AREDS), we examined redox states in the antioxidant and Zn2+ supplementation arms of this double-blind intervention design. Results showed that antioxidant supplements protected against an age-associated oxidation of Cys/CySS redox state in patients with age-related macular degeneration (AMD). In contrast, Zn2+ supplementation was not associated with redox change but was associated specifically with a decrease in plasma CySS concentration. Zn2+ supplementation protected against progression of AMD independently of antioxidant supplementation. Recent literature research shows that Zn2+ acts through Nrf-2/ARE to induce xc-, an important system for transporting CySS from plasma into cells. Because this system also induces expression of proteins for GSH synthesis, increased dietary Zn2+ intake can alter the metabolic utilization of plasma CySS to support increased GSH synthesis. Consequently, dietary Zn2+ intake may be of general importance in protection against chronic and age-related toxicities associated with oxidative stress and reactive electrophilic chemicals by enhancing tissue utilization of cystine for GSH biosynthesis.

S 2329 ZINC TRANSPORTERS AND THE REGULATION OF INTRACELLULAR ZINC TRAFFICKING.

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Sponsor: J. Kang.

Zinc is an essential nutrient because it is a required catalytic and/or structural cofactor of many different proteins. In contrast, zinc can also be toxic to cells if intracellular levels rise to too high a level. Thus, cells of all organisms have evolved with mechanisms to maintain intracellular zinc homeostasis as extracellular zinc availability changes. The molecular mechanisms of zinc homeostasis will be the subject of this presentation. In the yeast Saccharomyces cerevisiae, zinc uptake is mediated by three different transporters located in the plasma membrane. These transporters, Zrt1, Zrt2, and Fet4, are tightly controlled at the transcriptional level by the Zap1 zinc-responsive transcription factor. In addition to these genes, Zap1 regulates the expression of as many as 76 other genes in the yeast genome and we are probing the role of these genes in zinc homeostasis and the adaptation of cells to low zinc growth. These studies have shown that in addition to increasing zinc uptake abilities, cells respond transcriptionally to low zinc to mobilize intracellular stores of zinc and to increase the capacity for zinc transport into the secretory pathway to maintain function of those compartments. In addition, we have discovered a mechanism of isozyme switching in which the cell switches from the reliance on some abundant zinc-dependent enzymes, i.e. alcohol dehydrogenases, to isoforms that use other metal cofactors for their activities. Thus, the cells reduce their dependence on zinc. As a final example, we have found that zinc-deficient yeast cells, like cells of other organisms, experience increased oxidative stress. To prevent cellular damage from these reactive oxygen species, Zap1 increases expression of a cytosolic peroxiredoxin, Tsa1, to eliminate excess oxidants. This mechanism represents an adaptive response to zinc limitation. Thus, through the regulation of gene expression by Zap1, yeast cells respond in many ways to maintain zinc homeostasis within the cytosol and organellar compartments and to adapt to conditions of low zinc growth.

S 2330 METALLOTHIONEIN REDOX CYCLE AND ZINC SIGNALING.

Y. Kang. *Medicine, University of Louisville School of Medicine, Louisville, KY.*

Redox zinc switch is a recently recognized mechanism of regulation of conformational and functional changes of many important redox-sensitive proteins. In response to oxidative stress, zinc release from redox sensors causing functional alterations of the zinc-binding proteins and from redox transducers to alter the function of other proteins. Metallothionein (MT) contains high levels of sulfur. The mutual affinity of sulfur and transition metals makes the binding of zinc to MT thermodynamically stable. Under physiological conditions, zinc-MT is the predominant form of the metal-binding protein. Oxidation of the thiolate cluster by a number of mild cellular oxidants causes zinc release and formation of MT-disulfide (or thionin if all metals are released from MT, but unlikely occurs in vivo), which have been demonstrated in vivo. Therefore, the thermodynamic stability of zinc binding makes MT an ideal zinc reservoir in vivo and the redox regulation of zinc mobilization enables MT function in zinc homeostasis. MT disulfide can be reduced by glutathione in the presence of selenium catalyst, restoring the capacity of the protein to bind zinc. This MT redox cycle plays a crucial role in MT biological function and is linked to the converting of redox signals to zinc signals.

S 2331 REGULATION OF METAL-INDUCIBLE TRANSCRIPTION BY A ZINC-FINGER TRANSCRIPTION FACTOR.

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Upon exposure to metals, MTF-1, a 6 zinc-finger, metal-responsive transcription factor, translocates into the nucleus and binds to metal responsive elements (MREs). Subsequently, transcription of metallothioneins (MTs) and other genes occurs. There are two models that describe how metals affect MTF-1 to activate transcription. The first proposes that zinc binds to the zinc fingers, which results in a conformational change allowing MTF-1 to bind to MREs and activate transcription. The second proposes that metals affect signal transduction pathways that alter the level of MTF-1 phosphorylation, which controls its transcriptional activity. To investigative these models, mass spectrometry; to identify the phosphorylation sites; and site-directed mutagenesis; to determine the functional role of different domains of MTF-1 in controlling its activity; were used. MTF-1-GST was incubated with JNK-1 and -2, casein kinase II, or protein kinase C. Autoradiography confirmed that MTF-1 could be a substrate for these kinases. Initial analysis using MALDI-TOF and ESI mass spectrometries confirmed the presence of a phosphoserine in a PKC consensus sequence. Prosite analysis predicts 9 PKC phosphorylation sites in MTF-1. A collection of targeted, site-directed MTF-1 mutants inwhich all of the potential PKC phosphorylated residues have been modified has been generated. The ability of the mutant MTF-1's to activate transcription in response to cadmium and zinc is being assessed. Using a heterologous expression system, MTF-1 was expressed in yeast containing an MRE-based reporter gene. This system was used to screen randomly mutagenized MTF-1. More than 100 unique loci were modified and subsequently tested. Forty-five loci that are located within the zinc-fingers were shown to produce gain- or loss-of-functions. Approximately, 25% of the loss-of-function mutants retain wild type MRE binding affinity, which indicates that DNA binding is not synonymous with transcriptional activity, and that these loci are related to an alternative trans-activation mechanism.

S 2332 PERINATAL EXPOSURE TO NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS (NRTIS) INDUCES TRANSPLACENTAL GENOTOXICITY AND MITOCHONDRIAL TOXICITY.

M. C. Poirier. *National Cancer Institute, Bethesda, MD.*

Highly active antiretroviral therapy (HAART), containing two NRTIs, has been remarkably beneficial in managing HIV-1 infection and AIDS. However, increasing evidence suggests that in some HIV-1-uninfected children born to HIV-1-infected mothers, perinatal NRTI exposure may have long-term genotoxic and mitochondrial consequences. Recent studies, compiled in a special issue of Environmental and Molecular Mutagenesis (Vol. 48, April/May 2007), support earlier conclusions that AZT is a transplacental carcinogen in mice, becomes incorporated into DNA of fetal mice, humans and monkeys, and induces mutagenesis (including p53 transversion mutations) and micronuclei in mice and humans exposed to NRTIs in utero. Currently evidence for increased malignancy in HIV-1-uninfected children born to HIV-1-infected mothers who received NRTI therapy during pregnancy is lacking, but widespread use of NRTIs in human pregnancy has been recommended only since 1994. An additonal area of concern is mitochondrial compromise, which occurs in infants exposed to NRITs in utero. Similar morphological and biochemical evidence of NRTI-induced mitochondrial damage was found both in human infants born to HIV-1-infected mothers and monkey infants born to immunodeficiency virus-free dams given human-equivalent NRTI protocols. Therefore, transplacental drug treatment alone is able to induce fetal mitochondrial toxicity. Substantial morphological and biochemical mitochondrial cardiac damage was also seen in the NRTI-exposed infant monkeys, consistent with evidence of a deficiency in human cardiac left-ventricular mass reported for HIV-1-uninfected infants at 1 and 2 years of age after in utero exposure to NRTIs. This symposium will include 4 speakers who will cover topics related to exposures in animal models and parallel events in HIV-1-uninfected human infants born to HIV-1-infected mothers receiving NRTI therapy during pregnancy.

S 2333 OVERVIEW OF RODENT TRANSPLACENTAL CARCINOGENICITY AND GENOTOXICITY STUDIES INVOLVING NRTIS.

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Nucleoside reverse transcriptase inhibitors (NRTIs) have achieved unprecedented success in preventing the transmission of HIV from infected mothers to their infants. However, this success is tempered by evidence that these drugs interact with

host cell DNA resulting in the induction of persistent chromosomal alterations and mutations in both nuclear and mitochondrial DNA. The significance of these changes is underscored by the findings of increased cancer incidence in transplacental bioassays using rodent models exposed to the prototypic NRTI, zidovudine (AZT), during gestation. The first of these studies was designed based upon the assumption that the daily dose of nucleoside analog would be predictive of long term effects; this study found no evidence of increased incidence of neoplasms from transplacental AZT exposure. Subsequent studies designed upon the assumption that the cumulative dose would be a better indicator of long term effects, found increased incidences of neoplasia in liver, lung, reproductive tissues, endothelial cells (male B6C3F1 mice) and mononuclear cell leukemia (in female rats). AZT-treated female rats also had an increased incidence of brain tumors (gliomas) above concurrent and historical control animal values. Changes in indicators of tumor cell behavior were also observed in one study. This presentation will review the results of the transplacental cancer bioassays of AZT that have been completed to date and will also summarize results of genetic toxicology testing conducted to evaluate perinatal exposures of humans and rodent models to AZT and other NRTIs.

S 2334 MUTATIONS IN CANCER GENES IN LUNG TUMORS FROM SWISS (CD-1) MICE EXPOSED TRANSPLACENTALLY TO AZT AFFECT SIMILAR MOLECULAR PATHWAYS THAT ARE IMPORTANT IN HUMAN LUNG CANCER.

R. Sills. *NIEHS, Research Triangle Park, NC.*

In a transplacental carcinogenicity study, pregnant Swiss (CD-1) mice were exposed to 0-300 mg AZT/kg bw/day for the whole of gestation. Lung tumor incidences in the 200 and 300 mg AZT/kg male treatment groups were significantly greater, compared to the unexposed controls, and the lung neoplasms were evaluated for point mutations in the K-ras and p53 cancer genes. K-ras mutations were detected in 66% of the AZT-induced tumors, and the predominant mutations were codon 12 G to T transversions. p53 mutations were detected in 84% of the AZT-induced tumors, and the predominant mutations were exon 8, codon 285 A to T transversions, and exon 6, codon 198 T to A transversions. There were no mutations in five tumors from unexposed mice. The mutation patterns found in the lung tumors suggest that incorporation of AZT or its metabolites into DNA may contribute to the lung cancer incidence in these mice. Current cumulative data in mice and humans suggest that infants exposed transplacentally to AZT may be at increased risk for cancer as they age.

S 2335 PERINATAL NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR (NRTI) EXPOSURES CAUSE PERSISTENT MITOCHONDRIAL COMPROMISE IN MOUSE AND MONKEY OFFSPRING AND HUMAN INFANTS.

R. L. Divi[1], M. C. St. Claire[3], D. M. Walker[2], V. E. Walker[2] and M. C. Poirier[1]. [1]*NCI, NIH, Bethesda, MD,* [2]*LRRI, Albuquerque, NM and* [3]*BioQual, Rockville, MD.*

We have investigated mitochondrial toxicity induced by perinatal NRTI exposures in mice and non-human primates in the absence of HIV-1 infection, and in infants born to HIV-1-infected mothers. In B6C3F1 mice, *in utero* exposure to NRTIs caused mitochondrial ultrastructural pathology, mitochondrial loss, abnormal echocardiogram, increased heart size, mutations in mitochondrial tRNA genes and elevated cytochrome *c* oxidase activity. These indications of mitochondrial compromise were present at birth and persistent at 18 and 24 months of age. In *Erythrocebus patas* monkeys, we gave human-equivalent doses of NRTI combinations for the last 50% of gestation and to the offspring for 6 wk after birth. NRTI-exposed fetuses, though clinically asymptomatic, were born with mitochondrial morphological damage, mitochondrial DNA (mtDNA) depletion and oxidative phosphorylation (OXPHOS) enzyme activity alterations in heart and skeletal muscle. At 1 year of age, the mitochondrial morphological abnormalities persisted and there were abnormal quantities of mtDNA, while the OXPHOS activities improved. A comparison of mitochondria in umbilical cord endothelial cells and peripheral leukocytes from NRTI-exposed monkeys and human infants revealed similar mitochondrial morphological damage and mtDNA depletion in both species at birth. In addition, mtDNA depletion was persistent for at least 2 years in NRTI-exposed human infants and 1 year in exposed patas offspring. Overall, the data from NRTI-exposed mice and monkeys showed persistent mitochondrial damage in perinatally-exposed offspring, suggesting the potential for progressive functional abnormalities. The high degree of similarity for mitochondrial compromise observed in mice, patas and children exposed perinatally to NRTIs suggests that human infants should be followed long-tem for mitochondrial integrity and that research should focus on developing strategies for mitochondrial protection.

S 2336 THE EFFECT OF ART EXPOSURE DURING CHILDHOOD ON CARDIOVASCULAR STRUCTURE AND FUNCTION IN CHILDREN BORN TO HIV INFECTED MOTHERS.

S. E. Lipshultz. *Department of Pediatrics, University of Miami, Miami, FL1, Miami, FL.* Sponsor: V. Walker.

The effect of antiretroviral (ART) therapy exposure in utero or after birth is largely unknown. CHAART I and CHAART II are multicenter studies to examine the effects of antiretroviral exposure on cardiovascular structure and function in children. The CHAART I study has focused on transient ART exposure during the fetal and neonatal periods on subsequent cardiovascular structure and function during childhood. The CHAART I study has found that there are significant differences between these children and those followed on the NHLBI P2C2 study who were not exposed to ARTs. The significant differences are that the ART-exposed children have significantly less left ventricular mass and wall thickness after birth than those who were not exposed to ARTs and these significant differences persist during 2 years of postnatal follow-up. This reduced LV mass is associated with increased left ventricular afterload, a measure of stress on the wall of the left ventricle. In contrast to the reduced LV mass and wall thickness and the increased LV afterload the ART-exposed infants have more normal left ventricular systolic function as measured by fractional shortening and also of left ventricular contractility, a marker of the health of the cardiac muscle cells. Thus, it appears that maternal ART exposure results in healthier HIV infected mothers than those without ART therapy and that fetuses developing within healthier HIV infected women have more normal left ventricular function during the neonatal and infant periods but their LV mass and wall thickness are less than those found in children without ART exposure. Relationships to maternal health, biochemical markers of cardiomyopathy, and mitochondrial DNA mutations will be presented.
The NHLBI CHAART II study examines the long term use of ARTs during childhood in HIV infected children. This study will focus on whether the length of ART exposure or specific ART combinations are more toxic to the developing myocardium by assessment of left ventricular structure and function.

S 2337 STEM CELLS IN DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY AND TOXICOLOGY - SYMPOSIUM OVERVIEW.

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There is considerable concern that chemical exposures near the time of conception or during pregnancy may contribute to the increasing incidence of congenital cardiovascular and neurodevelopmental diseases. However, most chemicals have not been well characterized because comprehensive testing may exceed available resources of time, money and animals. While the embryonic stem cell test with morphologic and molecular endpoints offers an alternative strategy for screening pharmaceuticals and novel compounds, it is just one application of stem cells in toxicology. Adapting these cells to high through-put platforms may improve efficiency and offer reductions in animal use and costs. This vision is challenged by the long-term stability of the pluripotent stem cell populations and standardizing conditions for directed differentiation. Resolution of these issues is needed to fully capitalize on stem cells in developmental and reproductive toxicology. The purpose of this symposium is to provide an overview of stem cell biology and convey the current state-of-the-art science of using embryonic stem cells in developmental studies and toxicity testing.

S 2338 STEM CELLS IN REPRODUCTIVE AND DEVELOPMENTAL BIOLOGY.

D. P. Wolf. *Oregon National Primate Research Center, Oregon Health & Science University, Beaverton, OR.* Sponsor: A. Greenlee.

Stem cells have the unique ability to self replicate indefinitely or undergo differentiation depending on environmental conditions. While adult stem cells are multipotent, embryonic stem cells (ESCs), derived from the inner cell mass of mammalian blastocyst-stage embryos are the most robust of stem cells capable of giving rise to the three major cell lineages and all of the cell types in the body, including germ cells. ESCs, initially isolated in mice are available in primates; rhesus and cynomolgus monkeys and humans. This presentation will focus on the current status of research with primate ESCs. A molecular level understanding of 'stemness' is clarifying with regards the timing and specific expression of unique genes and proteins. Additionally, important insights concerning the genetic and epigenetic integrity of ESCs are now available. ESCs will enable investigations into how different cell-types are derived. On the applied side, the potential for human ESCs to be used in regenerative medicine remains an exciting possibility based on the premise that

many human diseases resulting from defects in a single cell type can be treated by cell or tissue replacement therapy. In anticipation, ESC derivation and maintenance under xeno-free conditions is required along with directing differentiation protocols yielding specific phenotypes. Purification and characterization of such populations of progenitor/terminally differentiated, functional cells precedes preclinical trials. Strategies to cope with the likelihood of immune rejection of ESC-derived phenotypes include therapeutic cloning (TC) whereby isogenic ESCs are created by superimposing somatic cell nuclear transfer on stem cell isolation. Thus, a nuclear donor cell recovered from a patient would be combined with a DNA/spindle-free oocyte to create a reconstituted embryo that would be activated, developed to the blastocyst stage and used for ESC isolation. Proof of principle for TC has been accomplished recently in the rhesus monkey. The use of embryonic stem cells in drug discovery and screening and toxicity evaluation will also be considered.

S 2339 METABOLOMICS OF HUMAN EMBRYONIC STEM CELLS: INNOVATION FOR DISCOVERY OF PREDICTIVE BIOMARKERS OF DEVELOPMENTAL TOXICITY.

G. G. Cezar. *Animal Sciences, University of Wisconsin, Madison, WI.* Sponsor: A. Greenlee.

The limitation of animal models to predict toxic outcomes of pharmaceuticals in humans is a major contributor to compound attrition and the soaring costs of drug development. In this presentation, I will discuss how human embryonic stem (hESC) cells may serve as a robust resource to predict developmental toxicity of drug candidates and to elucidate mechanisms of compound toxicity to specific human lineages, such as brain precursors and neurons. Biochemical pathways of human development are active in hESC and derivatives, since they recapitulate neurogenesis in vitro. Metabolomics is a new generation "omics" platform that measures the comprehensive set of small molecule metabolites in cells and tissues, allowing investigators to identify multiple pathways and networks that underlie toxic phenotypes. These "metabolic profiles" also serve as predictive biomarkers to screen for potential adverse effects of novel compounds to human development. Our pioneering studies have validated metabolomics of hESC as a means to discover candidate biomarkers for known developmental disruptors, such as valproate and alcohol, which will be reported in more detail. The establishment of a renewable cellular substrate with functional properties of in vivo counterparts for compound screening should greatly enhance predictive safety assessment of chemicals.

S 2340 STEM CELLS FOR DEVELOPMENTAL TOXICITY TESTING: THE PROMISE AND PERILS.

D. B. Stedman, J. A. Paquette, R. D. Streck, S. W. Krumpf and R. E. Chapin. *Global Research and Development, Pfizer, Groton, CT.*

The quest for an in vitro tool to predict developmental toxicity has been ongoing for nearly two decades. A number of model systems have been explored: rodent whole embryo culture, micro mass culture, FETAX, cell-cell communication, hydra and more recently Zebrafish. Because embryonic stem cells have the ability to undergo differentiation to all cell lineages and to some extent recapitulate early development, they may provide an even better tool for the study of developmental toxicity. Recently, The European Centre for the Validation of Alternative Methods (ECVAM) has validated an Embryonic Stem Cell Test (EST) for classifying developmentally toxic compounds. The overall accuracy of this assay in our hands is ~75% after evaluating 65 compounds comprising both chemicals and pharmaceuticals. While these results are similar to ECVAM's (78%) and as an in vitro predictive model are not bad, it does leave room for improvement. The EST assay clearly was not designed to separate within toxicity classes, and appears to have difficulty correctly classifying biologically active compounds that are cytotoxic at very low concentrations. It also has trouble distinguishing between compounds at the interface between non and weak embryotoxicity. These features are critically important for any developmental toxicity screening assay in a pharmaceutical environment. We are currently exploring adaptations of the EST assay, along with gene expression analysis of the embryoid bodies generated by the assay to provide additional endpoints. By incorporating these new measures, we hope to build an improved predictive model.

S 2341 EGGS AND SPERM FROM EMBRYONIC STEM CELLS: EMERGING TOOLS TO PREDICT REPRODUCTIVE RISKS?

A. R. Greenlee[1, 2]; [1]*School of Nursing, Oregon Health & Science University, La Grande, OR and* [2]*Center for Research on Occupational and Environmental Toxicology, Oregon Health & Science University, Portland, OR.*

Animal and human studies suggest contaminant exposures, at concentrations found in daily life, may impair gamete development in utero, compromise oocyte and sperm maturation, accelerate declines in ovarian reserves and reduce embryo survival. Minimizing exposures to reproductive hazards will depend on gaining a better understanding of links between contaminant exposures and adverse outcomes: i.e., one has to know what to avoid and when to avoid it. Biomarkers are needed for detecting early changes in male and female reproductive function, when interventions may have their greatest impact. Towards both objectives, new tools are emerging: the possibility of generating gametes from embryonic stem cells for toxicological assessments. In vitro differentiation of stem cells to eggs and sperm opens a previously inaccessible physiologic window for toxicology and biomarker studies. Human gametes from human embryonic stem cells (hESCs) could best represent human responses to reproductive toxicants. Gene-environment effects could be assessed using eggs and sperm derived from genetically defined hES cell lines. Ethical and political hesitation might be minimized by obtaining stem cells from alternative sources (e.g., amniotic fluid or bone marrow). Known challenges to this model include low efficiencies of gamete production and uncharacterized culture conditions that simulate the in vivo germ cell niche. The merits and limitations of generating gametes from embryonic stem cells and potential uses of this technique in screening reproductive hazards will be placed in context of recent scientific literature.

W 2342 GENOTOXICITY TESTING FROM EARLY DISCOVERY THROUGH REGULATORY SUBMISSION: A COMPREHENSIVE PRIMER.

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A review of the Physician's Desk Reference (PDR) reveals that 25-30% of marketed drugs (excluding those whose mechanism of action is dependent on genotoxicity such as some antineoplastics, antivirals, etc) exhibited genotoxicity when assessed by the standard battery of bacterial and mammalian assays. The majority of these marketed drugs with positive gene-tox findings were discovered and developed some years ago in a more relaxed climate in which genotoxic liability was balanced against the potential benefit of the indication. While this risk/benefit analysis still comes into play today, increased drug safety accountability has resulted in a "zero tolerance" situation thus mandating better ways to screen for and identify these problematic structures as early as possible. Most large pharmaceutical companies with active chemistry and/or high throughput combinatorial or compound library screening programs have recognized the need for ascertaining the genotoxic potential ofnovel molecular scaffolds have genotoxic potential and have established biological and in silico strategies to determine this. The goal of this workshop will be to provide an assessment of the state of the art of genotoxicity evaluation in the drug discovery process, the management of genotoxicity issues and regulatory accountabilities. The following topics will be discussed: 1) Strengths and limitations of existing traditional and scaled-down approaches. 2) Appropriate use of in silico programs for predicting genotoxicity including discussion on why these sometimes generate "false positives" and "false negatives" and new ways to look at these findings. 3) Genotoxicity associated with non-covalent drug/DNA interactions. 4) high throughput applications. 5) Novel genomics or other molecular-based approaches for genotoxicity assessment. 6) Future trends and regulatory expectations.

W 2343 GENETIC TOXICOLOGY TESTING: HOW CAN WE IMPROVE ITS UTILITY TO INFORM RISK ASSESSMENT?

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Genotoxicity testing plays a critical role during the discovery and advancement of new molecules for commercial applications involving human exposure. These tests range from *in silico* structure-activity relationships and high/medium throughput *in vitro* screens to short-term "guideline studies" to meet regulatory requirements. The identification of genotoxic potential of unique metabolites generated either in humans or in the environment is often facilitated either by the incorporation of relevant enzymatic systems in the *in vitro* assays or by testing the metabolite in question in a pure form. Genetic toxicology tests are primarily used for the identification and classification of putative animal carcinogens and, in the European Union, for labeling of chemicals for germ cell mutagenicity. A number of initiatives are underway to update the genetic toxicology testing paradigm in order to enhance its utility for human risk assessment instead of simply using the data for hazard identification. These initiatives include the identification of follow-up strategies to get a better insight into the significance of results from the initial battery of tests and the development of a weight of evidence approach for evaluating genotoxicity data. There are still ample opportunities to improve the design of genetic toxicology studies such that our ability to interpret findings from these tests is enhanced. It is suggested that results from genetic toxicology tests be interpreted not in isolation but in the context of all other toxicology data including dose-response relationships/no-observed adverse effect levels, mechanistic information, toxicokinetics, and relevant exposure data.

W 2344 USING GENETIC TOXICOLOGY INFORMATION TO EVALUATE SAFETY OF IMPURITIES AND METABOLITES FOR PHARMACEUTICALS.

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Prior to clinical development, drug candidates are assessed in standard nonclinical tests to determine their potential toxicity to humans. Impurities (including degradation products) and metabolites are qualified using nonclinical studies for the drug substance/product. These tests may not be sufficient for genotoxic compounds because of their carcinogenic potential. This session will discuss current practices for the safety assessment of genotoxic impurities and metabolites. For genotoxic impurities, safety assessments involve risk-based practices previously applied by the food industry with adjustment in allowable exposure based on the duration of dosing in the clinical trial. To date, guidance on the safety assessment and qualification of genotoxic metabolites is limited. Draft FDA guidance recommends structural alerts and relative abundance calculations as triggers for genetic toxicity testing. Furthermore, genetic toxicology data and/or structural concerns could drive a request to conduct a carcinogenicity study for a drug metabolite. An alternative for qualification of known or potentially genotoxic metabolites will be introduced for discussion where risk is quantified using a retroactive risk management strategy for data normally generated in the conduct of nonclinical and clinical studies.

W 2345 ICH S2B, A STANDARD BATTERY FOR GENOTOXICITY TESTING OF PHARMACEUTICALS: A TEN YEAR PERSPECTIVE. HOW WELL HAS IT SERVED U.S., CAN IT BE IMPROVED?

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Results from carcinogenicity studies are generally not available for drugs until the time of approval. Many people, including healthy volunteers are often exposed to pharmacologically active doses of the drug before carcinogenicity results are available. FDA's Center for Drug Evaluation and Research uses results of genetic toxicology studies as a surrogate for carcinogenicity during the drug development phase (clinical trials). The current ICH genotoxicity test battery (ICH S2B) has been in place for ten years and experience suggests that the bacterial mutation assay and in vivo cytogenetics have good specificity with relatively low incidences of false positives. The in vitro mammalian cell assays (cytogenetic metaphase analysis and the mouse lymphoma gene mutation assay) have poor specificity; one quarter of all assays give positive responses. The ICH guidelines for genotoxicity testing of human pharmaceuticals are currently under maintenance with the goals of reducing the frequencies of irrelevant positive responses and reducing animal usage. Possible changes to the guideline include: elimination of the requirement for a positive control with every in vivo study, including genetic endpoints as parts of general toxicology studies, offering as an option the use of the in vitro micronucleus test, and for in vitro mammalian cell assays, reducing the highest concentration tested and/or changing the level of cytotoxicity required at the highest concentration. Also under discussion are possible alternate strategies where multiple genetic endpoints are assessed in vivo in place of an in vitro mammalian cell assay.

W 2346 *IN SILICO* APPROACHES FOR ASSESSMENT OF GENOTOXICITY IN DRUG DISCOVERY AND DEVELOPMENT.

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The evaluation of the toxicological safety of novel pharmaceuticals is an extremely important, albeit resource-intensive, process involving both in vitro and in vivo testing the conduct of which is thoroughly defined by Federal guidelines. Microarray technologies, for the first time, make it at least theoretically possible to predict acute and chronic organ toxicities from short term in vitro or in vivo dosing through evaluation of gene expression changes associated with drug treatment. It would seem that, owing to an extensive understanding of covalent drug/DNA interactions and their consequences, genotoxicity of novel molecules should be even easier to predict, perhaps using primarily in silico approaches that would require no actual testing at all. While we have made great strides in this direction, we remain frustratingly far away from this eventuality. A description of the various ways in which computational tools are being or could be employed will be presented. Moreover, the strengths and underlying weaknesses of current computational programs will be evaluated along with speculations as to how one might hope to improve their utility through consideration of, among other things, non-covalent DNA interactions.

W 2347 EMERGING SCIENTIFIC APPROACHES IN GENETIC TOXICITY TESTING.

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Genetic alterations such as mutation and/or chromosome damage are considered hallmarks of cancer. Thus the assessment of cancer risk associated with exposure to drugs and chemicals relies primarily on the genotoxicity testing battery followed by the carcinogenicity testing via the 2-year rodent carcinogenicity bioassay. In this paradigm, the genotoxicity testing battery enables relatively simple, rapid and inexpensive hazard identification, namely the chemical's ability to cause genetic damage in cells. The 2-year rodent carcinogenicity bioassay provides mainly an assessment of the risk associated with a chemical's exposure to cause cancer in animals. Although the link between genotoxicity and carcinogenicity is well documented, this relationship is complicated due to the impact of non-genotoxic mechanisms of carcinogenesis and by the nature of in vitro genotoxicity assays and endpoints. Thus, the development of novel scientific approaches bridging genotoxicity and carcinogenicity testing via understanding underlying mechanisms is essential. Here, an alternative testing paradigm that utilizes the emerging genomic and systems biology science is introduced. In principle, the approach consists of a single in vitro test system that addresses the relevant principal genetic lesions assessed in the current test battery followed with systems biology methods to evaluate genotoxic mechanisms for positive compounds. The single in vitro test system possesses higher throughput attributes and allows for an initial differentiation of genotoxic mechanisms i.e. DNA-reactive and DNA–non reactive. Subsequent evaluation of genotoxic mechanism via systems biology methods could then be followed up with a complementary in vivo assessment to establish mechanistic relevance at potential target tissues.

W 2348 INCORPORATION OF MODE OF ACTION INTO MECHANISTICALLY-BASED QUANTITATIVE MODELS.

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The biological processes by which environmental pollutants induce adverse health effects is most likely regulated by complex interactions dependent upon the route of exposure, dose, kinetics of distribution, and cellular responses. To further complicate deciphering these interactions, the majority of toxicological data has been developed using in vitro and in vivo models, adding to the complexity of extrapolation issues. Quantitative models are the next logical step to advance the current toxicological understanding of how pollutants induce both cancer and non-cancer health effects. Mechanistically-based quantitative modeling represents an avenue of research to identify and, in some instances, quantify uncertainties in relationship to mode of action (MOA) by which compounds elicit adverse health effects. If the MOA for an environmental pollutant is known, a model can be constructed utilizing experimentally-derived datasets that quantitatively represent the key events in the MOA. Conversely, if the MOA is unknown, a hypothesis-driven approach can be utilized to assemble the model and inform likely key events within the MOA(s) for which further experimentations can be designed to test the MOA hypothesis. An important limitation of biologically-based model development is the availability of empirical data to construct such models. It is conceivable that this type of approach would improve the efficiency of current research strategies by pointing to specific research needs and data gaps. Efforts to define the approach for creating mechanistically-based models will provide unique and useful tools for human health risk assessment. Implementation of such techniques will result in the use of science-based derivation of risk posed by exposure to environmental pollutants. The following presentations will highlight the current state of the science and future directions of biologically-based quantitative modeling. (The views expressed in this abstract are those of the authors and do not represent the policy of the U.S. Environmental Protection Agency.)

W 2349 USING GENE/PATHWAY/DISEASE ANALYSIS TO QUANTIFY CRITICAL DISEASE PATHWAYS.

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High-content screening (HCS) (e.g. gene chips) and high-throughput screening (HTS) offer great promise as tools to help identify critical targets related to environmental exposures and to quantify the dose-response relationship between the critical targets and the environmental exposure. But how do you combine this information in a reasonable way to identify the most promising targets, quantify them, validate their importance and then actually use them in a risk assessment? In this presentation, I will focus on one part of this problem; using HCS data to identify targets for HTS and using HCS data to validate pathway connections identified by HTS. Using an algorithm developed in our laboratory for linking genetic/genomic data with diseases through cellular pathways, we are able to identify the critical pathways associated with a disease process. By then looking qualitatively and

quantitatively at these pathways we can identify useful targets for HTS. Positive findings from HTS assays that target one part of a pathway can be validated by using HCS data to show the expected quantitative shift in the pathway.

W 2350 USING GENOMIC DOSE-RESPONSE MODELING TO INFORM KEY EVENTS IN A MODE-OF-ACTION RISK ASSESSMENT.

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Microarray technology has been widely accepted as an efficient and reproducible way to explore the gene expression changes involved in the regulation of biological processes. In toxicology, the ability to examine thousands of genes allows a broad survey of gene expression changes following chemical exposure. Despite the potential utility of the technology, the application of microarrays to dose-response assessment has been limited due to the lack of analysis methods that examine the dose-response information in a risk assessment context. In this presentation, we describe an approach that uses traditional benchmark dose methods to model genomic dose-response data. The benchmark dose methods are integrated with bioinformatic analyses to calculate dose estimates at which different cellular processes are altered. The combination of microarray technology with these analysis methods results in a unique risk assessment tool that provides both a comprehensive examination of potential key events in the mode-of-action for a chemical and reference doses at which these key events occur.

W 2351 USE OF MODE OF ACTION (MoA) INFORMATION IN BIOLOGICALLY BASED DOSE-RESPONSE MODELING FOR INORGANIC ARSENIC: DOSE-RESPONSE (DR) AND TIME-COURSE (TC) CONSIDERATIONS.

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The DR and TC characteristics of toxicological endpoints depend on (1) the chemical characteristics of the toxicant and, (2) the biological mechanisms that determine target site dosimetry (PK) and the toxicological response of the target site tissue (PD). Biologically based DR (BBDR) models minimize uncertainty in the prediction of dose-time-response surfaces by maximizing the use of information on PK and PD mechanisms. The PD component of a BBDR model consists of a series of key events that link the driving tissue dose with the regulatory endpoint (i.e., a MoA). In the case of iAs and cancer, candidate key events include though are not limited to binding to sulfhydryl groups, generation of reactive oxygen species, DNA damage, changes in the activities of DNA repair enzymes, and cytolethality. If the BBDR model is to accurately describe overall DR and TC behaviors, then the correct sequence or sequences of key events must be identified and, for each key event, adequate DR and TC data must be collected. When multiple candidate key events exist, the DR and TC studies will provide the basic information needed to sequence the events with respect to time and to rank them with respect dose. When multiple sequences of key events are plausible, the sequence or sequences occurring at the lower doses will be preferred for inclusion in the BBDR model. Identification of key events and their TC and DR behaviors depend on input from experts in iAs toxicology. The special data needs of BBDR models, as outlined above, in turn specify experimental designs emphasizing TC and DR that are not commonly reflected in the existing iAs database. This work thus illustrates how the longer range goal of refining risk assessment for iAs depends on close collaboration between experimentalists and computational modelers. Input from regulators is also crucial to ensure that the regulatory endpoints of highest priority are addressed in the model. *This work has been reviewed by EPA and approved for publication but does not necessarily reflect Agency policy.*

W 2352 BIOLOGICALLY- BASED DOSE RESPONSE (BBDR) MODEL FOR THE HYPOTHALAMIC-PITUITARY-THYROID (HPT) AXIS.

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In rodents, thyroid active environmental pollutants disturb the hypothalamic-pituitary-thyroid (HPT) axis by defined modes of action, resulting in a disease referred to as hypothyroidism. Many species, including humans, are potentially susceptible to hypothyroidism caused by exposure to environmental pollutants. Thyroid hormones are necessary for normal functioning of many body processes. In the euthyroid adult rat, we developed a biologically-based dose response (BBDR) model for the HPT axis to explore the effects of iodide deficiency and perchlorate on this endocrine axis. The BBDR model of the HPT axis (BBDR-HPT model) was constructed using published literature and laboratory derived data. The current BBDR-HPT model includes submodels for iodide, thyroid stimulating hormone (TSH), thyroid hormones (T4 and T3). Key features of the BBDR-HPT model include: 1) the primary negative feedback loop, 2) tracking of thyroidal iodide stores for use as thyroid hormones, 3) TSH regulation of both the sodium iodide symporter protein, and the formation and secretion of thyroid hormones, and 4) extrathyroidal metabolism of thyroid hormones. This presentation will briefly cover the development of the BBDR-HPT model for the euthyroid rat and iodide deficient -hypothyroid rat, and the linkage of a submodel for perchlorate with the euthyroid BBDR-HPT model via a specific mode of action (competitive blocking of thyroidal uptake of iodide) to simulate perchlorate induced disturbances in the HPT axis. This model is used to test the hypothesis that the primary mode of action for perchlorate induced disruptions in the HPT axis is by blocking uptake of thyroidal iodide. Additionally, TSH controlled adaptive responses of the HPT axis to perchlorate insult are examined. Finally, dose- response profiles of HPT axis disturbances are simulated for iodide deficient rats exposed to perchlorate to better understand the consequences of perchlorate ingestion in the iodide deficient rat.
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W 2353 CURRENT AND FUTURE USE OF MECHANISTICALLY-BASED MODELS AND MODE OF ACTION IN HUMAN HEALTH RISK ASSESSMENT.

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The Environmental Protection Agency's Cancer Guidelines emphasize the use of mode of action (MOA) information in risk assessment for environmental pollutants to better characterize the risk of adverse health effects to humans. In addition, when available, the use of a biologically based dose response model (BBDR) is generally preferred because empirical data is used to characterize the pharmacokinetic and pharmacodynamic relationships of the key events in a MOA rather than default assumptions. Currently, significant progress has been made in utilizing pharmacokinetic models to inform dose extrapolation across species and will be explored using examples from recent human health risk assessments. In comparison, pharmacodynamic or BBDR models are not as developed because they are heavily dependent upon MOA information, which is often inadequate for environmental pollutants. Therefore, a key question arises, "how can BBDR modeling efforts inform missing information (e.g. MOA)?" In part, iterative modeling processes can be utilized to test MOA hypothesis, identify research gaps, and generate data to parameterize the BBDR model. Model predictions could either provide agreement with empirical data representing key events in the MOA or provide a basis to reject the hypothesized MOA. These future steps must consider the use of relevant phenotypic measurement representing key events, the source and magnitude of model uncertainty, uncertainty derived from the MOA, and variation in physiological or biochemical parameters. Overall, research for deriving MOA information and empirical data for environmental pollutants should be planned, prioritized, and conducted by estimating the eventual contribution to BBDR modeling as a key objective. In turn, the development of adequate BBDR models would reduce uncertainty in human health risk assessment.(The views expressed in this abstract are those of the authors and do not represent the policy of the U.S. Environmental Protection Agency.)

W 2354 PULMONARY TOXICITY TESTING OF NANOPARTICLES.

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The unusual physcochemical properties of engineered nanmaterials raise concerns about adverse effects on biological systems and developing testing strategies to characterize the hazard potential of increasing number of nanomaterials has been a challenge faced by regulatory agencies, academia and industries. In 2005, a screening strategy was developed by an International Life Sciences Institute (ILSI) workgroup for hazard identification process of nanomaterial risk assessment. The ILSI review proposes a comprehensive array of in vitro and in vivo assays to investigate the toxicity of diverse nanomaterials in routes of entry and target tissues. In vitro tests of toxicity can provide a rapid and relatively inexpensive way to assess the potential toxicity of large numbers of untested nanoparticles. In vitro studies also allow specific biological and mechanistic pathways to be identified and tested under controlled conditions to provide initial data on comparative toxicity of nanomaterials of diverse size and shape. However, there are a number of inherent issues of in vitro test systems resulting in false positives and false negatives and recent studies have shown little correlations between in vitro and in vivo toxicity of some nanomateri-

als. Because of their small size, aerosolization of respirable nanomaterials is likely, either as singlet or as aggregated particles and exposure by the inhalation route is a major concern. Whereas inhalation is the preferred method of pulmonary exposure for hazard identification, a number of barriers exist for conducting inhalation studies with nanomaterials, and alternative exposure methods (e.g., intratracheal instillation, intratracheal inhalation, pharyngeal/laryngeal aspiration) have been used by various investigators. The purpose of this workshop is to bring together experts to discuss the usefulness of in vitro studies for hazard identification of nanomaterials and if alternative pulmonary exposure methods can be used to substitute for inhalation studies.

W 2355 COMPARING *IN VITRO* AND *IN VIVO* PULMONARY TOXICITY OF FINE AND NANOPARTICLES.

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In vitro screening techniques are integral in the successful development of nanotechnology. Because of the rapid rate of nanoscale synthetic products and their derivatives, it is difficult to test every generation of nanoparticles in an in vivo experimental design. However, the in vivo response to particulate matter is pertinent to the integration of nanotechnology into our everyday way of life. To this end, the studies presented in this talk assess the capacity of in vitro screening studies to predict in vivo pulmonary toxicity of several fine or nano-sized particles in rats, including carbon-based and metal oxide nanostructures. Each nanomaterial was analyzed for both physicochemical and relevant toxicological properties. In the in vivo component of the study, rats were exposed by intratracheal instillation to each of the materials. Following exposures, the lungs of exposed rats were lavaged and endpoints were measured at numerous time points post-exposure. For the in vitro component of the study, different culture conditions were utilized and the culture fluids were evaluated similarly. When considering the range of toxicity to particles tested, the comparisons of in vivo and in vitro measurements demonstrated little correlation, particularly when considering many of the variables assessed in this study, such as cell-types, culture conditions, time course of exposure, and measured endpoints. It seems clear that in vitro cellular systems will need to be developed, standardized, and validated (relative to in vivo effects) in order to provide useful screening data on the relative toxicity of inhaled particles.

W 2356 COMPARISON OF *IN VITRO*, PHARYNGEAL ASPIRATION, AND INHALATION RESULTS FOR SINGLE-WALLED CARBON NANOTUBES.

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Once contaminating iron is removed from single-walled carbon nanotubes, they do not generate reactive oxygen species in vitro and their cytotoxicity is relatively low. In addition, these purified SWCNT fail to activate cytokine production or phagocytosis in cultured macrophages. These in vitro results correlate with the low level of uptake of SWCNT by macrophages following pharyngeal aspiration and the lack of persistent inflammation. In contrast, in vitro assays of oxidant stress or macrophage activation fail to predict the progressive interstitial fibrotic response to pharyngeal aspiration of SWCNT. However, SWCNT significantly enhance proliferation and collagen synthesis by fibroblasts in culture. Therefore, care must be taken in recommending a single in vitro screening assay (oxidant stress) as predictive of biological activity for all nanoparticles. Inhalation exposure to SWCNT results in pulmonary responses (transient inflammation but rapid and progressive interstitial fibrosis) very similar to those following pharyngeal aspiration. This indicates that pharyngeal aspiration is a reasonable alternative to more costly and time consuming inhalation studies as long as care is taken to use relevant exposure concentrations and to avoid exposure to non-respirable size agglomerates of SWCNT.

W 2357 THE NEED FOR VALIDATION OF TOXICITY TESTS OF NANOPARTICLES.

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Short-term in vitro toxicity tests are recommended for nanoparticles as they can yield data rapidly and can provide important insights on the mechanisms of toxic endpoints. There are, however, well-documented drawbacks of the in vitro assays such as their relative simplicity and the high dose effects. Therefore, mechanistic pathways which are elucidated and positive results obtained from in vitro test systems need further validation in animal models. Results of a testing strategy will be discussed, involving cell-free, cellular and in vivo assays. In vivo validation of in vitro results requires careful consideration, and several in vivo methodologies both acute and long term will be evaluated, with a focus on respiratory effects.

W 2358 PULMONARY TOXICITY STUDIES IN RATS WITH THREE FORMS OF ULTRAFINE TIO2 PARTICLES BY INTRATRACHEAL INSTILLATION AND INHALATION EXPOSURES.

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The results of several lung toxicology studies in rats have demonstrated that ultrafine or nanoparticles (generally defined as particles in the size range < 100 nm) administered to the lungs produced enhanced inflammatory response when compared to fine-sized particles of similar chemical composition at equivalent doses. However, the common perception that nanoparticles are always more toxic than fine-sized particles is based upon a systematic comparison of only 2 particle-types, namely titanium dioxide and carbon black particles. Based upon intratracheal instillation and inhalation studies with nano quartz and nano zinc oxide particles, and ultrafine TiO2 particles of different crystal structures– we have demonstrated that surface reactivity rather than particle size or surface area plays a key role in producing pulmonary inflammation and other adverse effects. In another study we assessed the pulmonary toxicity of inhaled or intratracheally instilled TiO2 particle formulations with various surface treatments in rats. The particle formulations were identical for both studies. The results from these studies demonstrated that for both inhalation and instillation exposures, only the TiO2 particle formulations with the largest components of both alumina and amorphous silica surface treatments produced mildly adverse pulmonary effects when compared to the base reference control particles. The results demonstrate that the intratracheal instillation-derived pulmonary bioassay studies with TiO2 formulations represented an effective screening tool for inhalation studies.

IS 2359 REACH – IMPLEMENTATION, CHEMICAL SAFETY, AND INFORMATION REQUIREMENTS.

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In December 2006, the REACH Regulation (Registration, Evaluation, and Authorisation of Chemicals) was adopted by the European Union. As a part of this regulation, manufacturers and importers will be required to provide information concerning the safety of their particular chemicals/substances. The goal of this legislation is to compile information on the hazards, exposure, and risk from chemicals in commerce. To that end, registration of each chemical will require a suite of data on physicochemical and toxicological properties. Sharing of data between companies registering the same substance will be encouraged for most data, and mandatory for animal data. The amount of data compiled for each substance will be based on the mass that is manufactured or imported annually in the EU. Those chemicals determined to be the most dangerous will be banned from use in the EU. For substances manufactured or imported in amounts greater than 10+ tonnes per year, hazard and risk assessments are required as part of a Chemical Safety Report. These assessments are required for each identified use of the substances, and are expected to describe appropriate risk management procedures and technology. The identification of uses and exposure scenarios will require an unprecedented degree of communication up and down the supply chain. Implementation of REACH will require a significant effort on the part of the global chemicals industry, with a specific need for expertise in toxicology and risk assessment. Pre-registration will begin in June 2008 and continue until December 2008. Registration of substances manufactured or imported in quantities greater than 1000 tonnes per year, as well as the substances identified as the most carcinogenic, mutagenic, and toxic to reproduction, must be registered by December 2011. Phased deadlines for substances in other tonnage bands continue until 2018. The regulatory authorities in the EU are developing a comprehensive set of guidance documents, called REACH Implementation Projects (RIPs). These documents describe the regulatory process and the necessary IT infrastructure, as well as provide guidance on how to comply with the regulation. This information session will focus on details of the REACH regulation as well as the REACH Implementation Projects, which will offer guidance to regulatory authorities and to industry.

IS 2360 REACH IMPLEMENTATION PROJECTS: GUIDANCE FOR INDUSTRY AND REGULATORY AUTHORITIES.

E. S. Williams. *Houston, ChemRisk, Houston, TX.*

The REACH Regulation is accompanied by an array of guidance documents, known as the REACH Implementation Projects (RIPs). Regulatory authorities and industry representatives work together to develop these guidance documents. There are seven REACH Implementation Projects (REACH Process, REACH-IT, Guidance for Industry, Guidance for Authorities, Setting up the Pre-Agency, Setting up the Agency, and Commission preparation for REACH), with many subsections. Development of these documents is not expected to be completed until early- to mid-2008. RIP 3 provides guidance for industry on 10 topics including

registration of substances, preparation of chemical safety assessments, fulfillment of data requirements, sharing of data, participation of downstream users in the REACH process, naming and classification of substances, and compliance requirements for substances in articles. RIP 3.4, guidance for data sharing, will describe the formation of a Substance Information Exchange Forum (SIEF), the mechanism for sharing of data between potential registrants of substances. The final version of RIP 3.8, guidance for industry on registration of substances in articles, will provide decision-making guidance on REACH obligations for substances which are components of commercial objects. Guidelines are provided in RIP 3.10 on the proper procedures for naming well-defined substances and those of variable or unknown composition. RIP 4 offers guidance for regulatory authorities on dossier and substance evaluation, and development of the list of substances subject to authorization. In their final form, the RIP documents will be critical resources for toxicologists charged with REACH compliance, as well as those involved in risk assessment in the global community.

IS 2361 FULFILLING TECHNICAL REQUIREMENTS OF REACH.

S. Borghoff. *Investigative Toxicology, ILS, Inc., Research Triangle Park, NC.*

Fulfilling the technical requirements of REACH begins with compiling a standard set of data for a large number of commercial and industrial substances. The scope of data to be compiled is dependent on the amount manufactured or imported in the EU. Each registration dossier will include all available data on physiochemical characteristics, as well as toxicological and ecotoxicological properties. For the upper tonnage bands, the amount of data to be compiled is very extensive. However, if the data is unavailable for an upper tonnage band substance, testing proposals must be submitted for review prior to implementing a testing program to minimize unnecessary testing and use of animals. Testing requirements range from developmental and reproductive toxicity testing, in vivo mutagenicity studies, acute and subchronic toxicity tests, and with high tonnage compounds, long term repeated toxicity and carcinogenicity testing. An estimated 55% of testing costs will be directed at fulfilling requirements for developmental toxicity and two-generational reproductive toxicity testing. A summary of the information and testing requirements outlined under REACH to determine potential health effects of a substance will be presented along with the role of data interpretation, weight-of-evidence for hazard identification and the potential use for using validated alternative testing methods that can provide the same level of information as animal tests.

IS 2362 REACH'S COMPREHENSIVE APPROACH TO CHEMICAL SAFETY.

V. Santa-Cruz. *Chevron-Phillips Chemical, Brussels, Belgium.*

The new European Chemicals regulation, REACH, was adopted on December 18, 2006 and entered into force on June 1st, 2007. REACH stands for Registration, Evaluation, Authorization and Restriction of Chemicals. The regulation puts major responsibility on industry for ensuring the safe manufacture, import and use of chemicals throughout the chemical supply chain. Manufacturers and importers (M/I) are required to perform a chemical safety assessment (CSA) and complete a chemical safety report (CSR) for substances manufactured or imported in a quantity above 10 tonnes per year per registrant. The CSA of a substance shall use data compiled in the registration process to conduct the following: human health hazard assessment, physicochemical hazard assessment; environmental hazard assessment, and a persistent, bioaccumulative and toxic (PBT) and very persistent and very bioaccumulative (vPvB) assessment. These steps will be undertaken to address each identified use of each substance, and will cover the life-cycle of the substance from manufacture through disposal. The goal of these assessments is to determine a derived no-effects level (DNEL) for human exposure, as well as a predicted no-effect concentration (PNEC) thought to be protective of environmental systems, if such determinations are possible from the available data. If the registrant concludes that the chemical meets the criteria for classification as dangerous, the CSA shall include exposure assessment and risk characterization. An important component of the CSA is to evaluate and ultimately recommend risk management measures (RMMs) which are protective of human health. The information derived from the CSAs will also be used in classification and labeling of the substance, as well as safety data sheets. The importance of CSA development and communication in the supply chain requires an increased competence throughout industry and has prompted the development of new tools or enhancement of existing tools to support M/I and DU.

IS 2363 REACH EXPOSURE SCENARIOS, ASSESSMENT, AND TOOLS.

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The new EU Chemicals legislation - REACH - introduces a number of new concepts for those manufacturing and importing chemicals. One of the significant challenges they will meet is how to gather use and exposure data on substances. REACH requires that substances that are classified as dangerous within the EU are assessed for their risks to man and the environment. To do so it is necessary to develop Exposure Scenarios - a description of a mixture of use and risk management measures - that can be related to either exposure data or an exposure assessment; which is then used in the risk characterization process. If the risk characterization does not result in an exposure that leads to a conclusion of no risk to man and the environment, then the substance cannot be supplied to the market for the specific use. This will drive an iterative process to increase the knowledge and certainty of exposure data on key products and processes. It seems likely that exposure science, too long a neglected aspect of the risk assessment world, will emerge as a major aspect of the industry and activities. This increased focus on exposure data is a definite change and will require a new skill set within many companies. One of the current problems for those planning how to cope with the demands of REACH, is that the exact nature and extent of the exposure scenario is still under some debate in Europe. It is expected that the necessary guidance will become public before the end of 2007. Ongoing discussions in the EU will address the issues and outcomes from the technical guidance process, as well as other initiatives, and the actions that they will necessitate. As the REACH guidance and understanding of the Regulation evolve, exposure assessment methodologies and associated tools will be built to cope with the demands that REACH will bring - such as those to facilitate the communication of exposure scenarios that is required in both the new extended safety data sheet and within the Chemicals Safety Report.

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